

488
50

Psychological

Monographs

General and Applied

**Anoxia, Behavioral Development, and Learning Ability:
A Comparative-Experimental Approach**

By

Gilbert W. Meier

Vanderbilt University

Marion E. Bunch

Washington University, St. Louis

Carson Y. Nolan

American Printing House for the Blind

and Charles H. Scheidler

University of Dayton

Price \$1.50

74
1

Edited by Norman L. Munn

Published by the American Psychological Association, Inc.



Psychological Monographs: General and Applied

Combining the *Applied Psychology Monographs* and the *Archives of Psychology*
with the *Psychological Monographs*

NORMAN L. MUNN, Editor

Department of Psychology, Bowdoin College
Brunswick, Maine

Consulting Editors

ANNE ANASTASI
FRANK A. BEACH
ARNOLD M. BINDER
W. J. BROGDEN
ROBERT R. BUSH
JOHN F. DASHIELL
JAMES J. GIBSON
D. O. HEBB
EDNA HEIDBREDER
FRANCIS W. IRWIN

JAMES J. JENKINS
HAROLD E. JONES
DANIEL KATZ
BOYD McCANDLESS
DONALD W. MACKINNON
QUINN McNEMAR
LORRIN A. RIGGS
CARL R. ROGERS
RICHARD L. SOLOMON
ROSS STAGNER

Manuscripts and correspondence on editorial matters should be sent to the Editor. *Psychological Monographs* publishes comprehensive experimental investigations and programmatic studies which do not lend themselves to adequate presentation as journal articles. Major space is given to the author's original contribution; introductory and bibliographic materials, as well as statistical tables and graphs, must be kept within reasonable bounds. Tables, graphs, and appendix materials which deal with detail not essential to adequate presentation of the findings may be made available through the American Documentation Institute—for details of this procedure, see the *APA Publication Manual*. Preparation of manuscripts for publication as monographs should follow the procedure given in the *APA Publication Manual*. Publication in *Psychological Monographs* is free of cost to the author, except in cases where early publication is requested or author's alterations are made in galley proofs.

ARTHUR C. HOFFMAN, Managing Editor; HELEN ORR, Promotion Manager

Correspondence on business matters should be addressed to the American Psychological Association, Inc., 1333 Sixteenth St., N.W., Washington 6, D.C. Address changes must arrive by the 10th of the month to take effect the following month. Undelivered copies resulting from address changes will not be replaced; subscribers should notify the post office that they will guarantee third-class forwarding postage.

COPYRIGHT, 1960, BY THE AMERICAN PSYCHOLOGICAL ASSOCIATION, INC.

Psychological Monographs: General and Applied

ANOXIA, BEHAVIORAL DEVELOPMENT,
AND LEARNING ABILITY:

A COMPARATIVE-EXPERIMENTAL APPROACH

GILBERT W. MEIER

Vanderbilt University

CARSON Y. NOLAN

American Printing House for the Blind

MARION E. BUNCH

Washington University, St. Louis

AND

CHARLES H. SCHEIDLER

University of Dayton

DESPITE THE fact that it has been strongly suspected for nearly a century (Little, 1861) that severe anoxia during early development may be responsible for certain neurologic disorders and mental retardation in children who would otherwise be normal, the problem of determining the actual contribution of the anoxia to the abnormality of behavior has remained an exceedingly difficult one. Relatively recent attempts to study the question by controlled animal experimentation in the laboratory have had the advantages of more accurate knowledge and management of the oxygen deficiency to which the animals are exposed, but they have encountered the difficult question of the measurement of psychological processes which may be adversely affected.

The researches discussed in the following monograph have tried to evaluate the effect of oxygen deficiency prior to birth and at birth. They are the culmination of an organized program initiated in the Department of Psychology at Washington University which has been generously supported by grants from the National Institute of Mental Health, United States Public Health Service (M-362 and M-762), and the United Cerebral Palsy, Incorporated (PL-45). Essentially, these studies have shown that a physiological insult, such as anoxia, incurred during early development *can* have an effect on psychological processes, at least those involved in problem solving behavior. The mechanisms by which these changes are effected have not been elucidated. This elucidation is the purpose of further re-

search which, at this time at least, points to a connection with the investigations on the effect of early experience.

The studies reported upon here, along with those which have followed the program initiated at Washington University (Meier, in press; Meier & Menzel, 1955), illustrate a point of view which the authors (among others) feel needs re-emphasis at this time. Within the range of species of animal life adaptable to laboratory conditions, the similarities between them should prove more important than the differences. Certainly this is true for the understanding of *behavior* rather than behaviors, which are the unique presentations of a particular species.

Clearly, this idea is not new. In those other life sciences in which systems or parts of organisms are of prime interest, e.g., ecology, morphology, and physiology, this truism has been utilized, as well as realized, for some time. For psychology, the usage of a wide range of species offers obstacles of greater proportion than those apparent to the other sciences. Where the time of laboratory maintenance is long, which is the usual case in behavioral research, considerations of size, reproductivity, diet, physiological rhythms, and tractability must be made. In addition, as we have had to do here, the investigator may have to consider the methodology of testing behavior, including the design and complexity of problems available (or which could be feasibly offered), the nature of the growth curve, and the relative developmental stage at birth. All of these appear to be outside the

scope of the problem of immediate experimental interest even though the extent to which each or all of them are evaluated will, to some degree, determine the manner of evaluation and extent of generalization of the results and conclusions of the research.

As stated, the impetus for the original researches cited here were the clinical problems centering upon the lack of oxygen during early development. For these investigations, the cat and rat (and, more recently, the chick) were chosen as experimental media. These choices were dictated by the particular phase of the problem studied and the characteristics of the species. (With the recent studies involving the chick, the unusual technical expedient for prenatal deprivation was the major consideration.) We feel that these investigations involving a multispecies approach not only demonstrate the operational practicality of the approach itself, but make the results more tenable, generally, and indicative of behavior changes which are the outcome of a disruption of a sensitive metabolic balance in periods of maximal cellular differentiation and growth.

SOME ASPECTS OF THE PHYSIOLOGICAL MECHANISMS IN ANOXIA

A sketch of the principal physiological mechanisms involved in oxygen deprivation should provide some appreciation of the behavior variations discussed in this monograph. The choice of the premature organism delimits the scope of the topic that need concern us here.

The premature organism, in these studies the fetus and the neonate, is characterized by certain metabolic features markedly variant to those found in the mature subject.¹ First, and most obviously, the fetus and the neonate are capable of withstanding degrees of oxygen deficiency far greater than can the more developed animal. For example, rabbit fetuses 29 days after conception sur-

vived an atmosphere of pure nitrogen as long as 44 min. Under similar circumstances, the neonate survived 21 min., whereas the 19 day old infant survived only 1½ min. (Glass, Snyder, & Webster, 1944). Depriving chicken embryos and fetuses of oxygen, Meier (unpublished data) noted similar relationships. At the end of 1 day, the median lethal dose (LD_{50}) was 210 min.; at 4 days, 105 min.; at 8 days, 65 min.; at 12 days, 43 min.; at 16 days, 35 min.; and at 19 days, 25 min. In both researches, the ability of the young organism to survive the deficiency was considerably greater than that of the old. The results together suggest a negative exponential function; the ability decreases sharply with maturation.

The explanation of these differences in tolerance to an oxygen lack in the environment lies in the nature of the energy production at the various stages in development. In the early embryo the metabolism is entirely anaerobic; in the adult, the metabolism is largely aerobic. This is a recapitulation of phylogenesis in physiological terms in that with the increasing complexity of structure and function there occurs an increasing efficiency of energy mobilization. With reference to metabolism this has meant a change from anaerobic to aerobic utilization of nutrients in the production of energy; this offers the organism a twelve-fold increase in energy output (Himwich, 1951, p. 143).

Second, the limit on organismic survivability to oxygen lack is set, in part, by the peculiarities of the nervous system. The increasingly greater role of aerobic metabolism during development describes this system more aptly than any other in the body. Although at no time in the organism's life is this system completely without anaerobic metabolism, the dominance of aerobic over anaerobic increases rapidly, especially in the period immediately after birth. The arrival at the adult metabolic status depends on the species and its characteristic rate of maturation. In the rat this level is reached at 21 days after birth; in the dog comparable functioning occurs following the lapse of 35 to 40 days (Himwich & Fazekas,

¹ A fuller discussion of the relationships between maturation and metabolism will be found in Himwich's *Brain Metabolism and Cerebral Disorders* (1951).

1941). (For this comparison it is assumed that the dog is no more immature at birth than is the rat.)

It follows from the foregoing that primary consideration should be given to the physiological mechanisms of the nervous system. This should not be construed to be a theoretical bias on the part of the authors with regard to the importance of this part of the organism in the totality of its behavior. Insofar as oxygen and the physiology of the body is concerned, the nervous system is the most readily affected, in both the young and the old. When it ceases to function under abnormal conditions of availability of oxygen, eventually, so does the remainder of the organism.

Third, in addition to the uniqueness of the metabolic processes of the nervous system as related to the survivability of the organism, a further fact must be recalled: there are certain delaying actions on the resistance of the adult to physiological insult. This is the fact of the irreversibility of the developed nervous system once the proliferation of the neuronal cells ceases, shortly after birth. Although these cells may continue to grow in size or in the length of their fibrous processes, they do not multiply. Although there occurs a regeneration of parts of the individual cells, regeneration of parts of the system is absent. In terms of the viability and behavior of the organism, this means that in the older individual the debilitation following oxygen deprivation (or some other such insult) will be more extensive and less transitory. Further, artificial aids in the sustenance of life (as some sort of technique for inducing and continuing respiratory activity) will be effective only so long as they are in operation; expectations that the organism will regain the facilities to carry on these vital functions are futile. Lastly, death may follow the deprivation after an appreciable lapse of time because of the inability of the mature system to replace the integral cells which are now fully nonfunctional and degenerated.

Finally, besides the transition in the relative roles of the two forms of metabolism, an equally apparent transition is evidenced

by the physiological and behavioral importance given to specific portions of the nervous system at particular phases in development. In terms of the individual's development, the importance of each of the portions follows closely the patterns seen in phyletic growth. As indicated by the rate of differentiation and growth of the constituent cells, the level of oxygen consumption, and the rate of integrated function, the developmental hierarchy proceeds from the lowest centers in the medulla and cord to the highest in the diencephalon and cerebrum. The converse of this is depicted by the adult undergoing oxygen deprivation (by drugs, simulated high altitudes, etc.). The cortical functions are the first to cease; the medullary and spinal are the last.

For the birds and mammals the climax in the developmental hierarchy is not reached until after birth. Conversely, in the neonate, centers other than those in the cerebrum are functioning maximally; these are the midbrain, pons, and medulla. As indicated by electroencephalographic activity, a crude measure at best, the first appearance of fully integrated neural activity is in the last third or last quarter of gestation (Jasper, Bridgman, & Carmichael, 1937). At parturition, the activity is still slight, with irregular, slow frequencies and low amplitudes. In most forms the adult pattern is reached considerably later (in the human about 10-12 yrs.) (Charles & Fuller, 1956; F. A. Gibbs & E. L. Gibbs, 1950; Libet, Fazekas, & Himwich, 1941).

So far, the discussion has been upon the descriptive stages in early functional development. Each of these stages encompasses but a brief temporal interval. In such longitudinal researches, as in one reported in this series, these stages, individually, are of unknown significance as determined by the data at hand. That is to say, to date there is insufficient evidence by which one can competently describe the relation between the time of the physiological insult and the particular structures undergoing principal development or the sequelae in the realm of behavior dysfunctions. Possibly, there is a family of curves representing each of the structures, generally alike but each with a

different time scale. Each curve rises to a maximum at the period of greatest sensitivity to the insult, then maintains that high point (Levinson, 1952) or, for less complex systems, slowly falls (Ingalls, 1947a, 1947b; Meier, 1953). Physiologically, this is a description of the possible bases of the behavioral phenomena of "critical period" and "psychological irreversibility."

The relation between developmental age (as determined by the level of oxygen consumption) and the severity of effect shows no sign of confounding with specific morphological or functional deviations which could denote impairments in localized structures or systems. This poses an interesting, though still unresolved, problem. Through the stages of fetal and postnatal development, the center making the greatest metabolic demands may not be among those centers most adversely affected. Acute alternations in the young organism are not necessarily followed by appropriate chronic, lasting deviations.

Correlated behavioral-histological studies have born out this observation. Windle, Becker, and Weil (1944) have reported permanent histological sequelae to natal asphyxia as varied, with localized and diffuse degeneration. No relation with particular neural structures was apparent. Severity of deprivation and certain behavioral manifestations likewise appeared to be without concurrence. These animals (guinea pigs) had showed marked sensory-motor dysfunctions (and asphyxia) for the first few days following birth. These included tremors, spasticity, rigidity, somnolence, and paralysis. For the most part, these disappeared within a few days, whereupon the animal assumed the air of normalcy. (The histopathology immediately after deprivation, that is, within one to five days after birth, consisted of, largely, multiple, diffuse capillary hemorrhages.)

Observing only behavior changes, Meier and Menzel (1955) reported that certain changes in behavior did follow deprivation at specific periods in fetal growth. However, rather than use the amount of oxygen metabolized as an index for specified periods of development, they used growth

schedules based upon cell size and proliferation. In this experiment, chicken eggs were deprived of oxygen through immersion in water at incubation temperature. Growth schedules were those listed in Hamilton's (1952) *Lillie's Development of the Chick*. On the simple, visually oriented tasks used in the study, that group which had undergone deprivation when the visual-motor system was developing maximally (about the eighth day of incubation) demonstrated the greatest differences in behavior. Like the findings of Windle and Becker (1943), some of the differences disappeared within a week or so after hatching. Unlike their findings, however, differences appeared at this time which were not detected on the first behavioral tests. At this time other groups (those deprived at 12 and 16 days) were different from the controls. As suggested in this report, there may be a differential effect of the early posthatching experience.

Typically, increases in complexity of structure and function and in efficiency of action are related to increases in complexity of behavior need satisfaction and/or to increases in the number of environments compatible to the organism. Growth in any environment means a dependence of the organism upon certain features (moisture, oxygen, pressure, radiation, and temperature) necessary for normal development. Although these of themselves are not sufficient, any limitation in the balance of these features may halt the developmental process and, possibly, life as well. In any environment these features are not constant; to the organism, they vary capriciously. Although these physicochemical processes of development may not be balanced on a pinnacle, certainly they are balanced on a tiny plateau. The confines of this plateau are the limits of adaptability and, ultimately, survivability.

I. NATAL ANOXIA AND LEARNING OF RATS AT MATURITY

Previous studies (Armitage, 1952; Becker & Donnell, 1952; Hurder & Sanders, 1953; Meier & Bunch, 1950; Scheidler, 1953;

You
anox
equi
of s
this
utiliz
learn
playe
more
of re
perio
learn
tively
the p
Th
pothe
diatel
learn
levels
2.979
one h
were
maze
great
used.
this p
releas
I lea
Skinn
while
simple

One
were u
from 9
in Gro
condit
their h
No sig
existed
the 59
anoxic
later in
the col
Washin
racks o
cage. 1
ratory c

Depriv
The a
animals

Youngdahl, 1948) of the effects of natal anoxia on later learning have produced equivocal results. The present study is one of several directed at a re-examination of this problem. Past studies have generally utilized relatively simple problems to test learning differences among the groups employed. It was believed that (a) use of more complex learning tasks, (b) measures of retention of such tasks after short time periods, and (c) measures of resistance of learned responses to extinction might effectively demonstrate differences unrevealed in the past.

The present study tests the general hypothesis that deprivation of oxygen immediately following birth results in reduced learning ability for rats at maturity. Two levels of deprivation, one-half hour at 2.97% equivalent oxygen (Group I) and one hour at 2.97% equivalent (Group II), were used. The 14 unit Stone type water maze was believed to be a learning task of greater complexity than those previously used. Both groups learned and relearned this problem. Following the learning and relearning of the 14 unit water maze, Group I learned a bar pressing response in the Skinner box which was later extinguished, while Group II learned and reversed a simple position habit.

Subjects

One hundred and fifty-six animals from 18 litters were used in Group I of the study and 59 animals from 9 litters were used in Group II. Of the 156 in Group I, two died in the dessicator under anoxic conditions, and 43 (23 controls, 20 anoxic) died in their home cages before reaching 60 days of age. No significant differences in the mortality rates existed between the groups during this time. Of the 59 in Group II, 10 died in the dessicator under anoxic conditions, and 2 (1 control, 1 anoxic) died later in the home cages. All animals were from the colony of the Department of Psychology, Washington University. They were housed in the racks of metal cages, three to four animals to the cage. The standard colony diet was Purina laboratory chow and water.

Apparatus and Procedure

Deprivation

The apparatus used to produce anoxia in the animals consisted of a vacuum bell jar with a small

vacuum pump attached. A manometer measuring from 0 to 90 cm. of mercury was attached to indicate the air pressure within the bell jar. The amount of air flowing through the system was regulated by a stopcock, the adjustment of which allowed airflow through the chamber at all times so as to prevent stagnation of air within the chamber itself.

Through close observation of pregnant females near term, newborn litters were obtained for use within three hrs. of birth. The criterion for rejection of litters was suckling: those litters of animals which showed milk in the stomach were rejected. Acceptable litters were removed from the maternal cage, divided randomly in half, and those animals designated as controls replaced. The experimental animals were marked by excising some of the tissue of the left earbud.

Following marking, they were immediately subjected to an environment of reduced oxygen content. This was accomplished by placing the animals in the bell jar from which the air was gradually withdrawn over a 10 min. period. At the end of this time a difference in pressure of 66 cm. of mercury existed between the inside of the chamber and the outside air. This environment of less than 3% equivalent oxygen was maintained for a period of 30 min. for animals in Group I of the study and 60 min. for animals in Group II. At the end of the appropriate period the pressure was returned to normal; this process also consumed 10 min. Fatalities during deprivation were less than 1% for Group I and 31% for Group II. The animals were returned to their mothers and reared normally. Littermates were later further differentiated by notching the right ear.

Behavior Measures

A straight pathway tank was used to accustom the animals to swimming. This tank was 108 in. long, 15 in. deep, and 8.5 in. wide. The depth of water in the tank was 9 in. At the end of the tank was a wire mesh ladder which led to an enclosed platform.

Animals were given an initial swimming experience between 65 and 72 days of age. This range of age differences was necessary in order to run each litter as a group. The initial swimming experience consisted of five trials per day in the straight runway tank for each of five days.

A 14 unit water T maze of the Stone type was used to measure learning. This apparatus has been previously described by Licklider and Bunch (1946). A wire mesh ladder at the end of the maze led to an enclosed compartment in a revolving horizontal drum where the animals were housed between trials. The temperature of the water in the maze was approximately 70° F. at all times. The water filled the maze to a depth of about 9 in.

Maze training for all groups was started the day following the finish of practice swimming. The animals were run 18 massed trials per day in

the multiple T maze until a criterion of one errorless trial was reached. The motivation used was escape from water. Each trial was scored for errors. An error was defined as entrance of the rat's head and shoulders into a blind alley. Time between trials varied depending on the number of animals in the litter and the degree of learning of the animals. Time between trials was never more than 65 min. (in the early stages of learning) or less than 5 min.

Thirty days following the attainment of the criterion for initial learning, the animals were required to relearn the maze. Running conditions were as previously described. The criterion for relearning was again one errorless trial.

Two standard type Skinner boxes were employed with Group I. To each was attached an electric counter which recorded the number of times the bar was pressed. The boxes were adjusted so that pressure on the bar resulted in the sound of the buzzer which was immediately followed by release of a pellet composed of 50% Purina laboratory chow and 50% sugar.

Pretraining for the Skinner box was carried out in a "pseudo" Skinner box. The interior of the "pseudo" box was identical to the Skinner box employed except that it did have a stationary food tray but did not have the bar.

At 150 days of age pretraining for the Skinner box was started for Group I. The animals were placed on a 23 hr. hunger schedule. On each of the 7 consecutive days access to food of the type used for reward in the Skinner box was allowed in the home cage for a one hr. period. Water was available at all times. Three days of training were next given in the "pseudo" Skinner box. During this period the animals were allowed to eat for an hour each day from the food trays of the training box. Their entire daily ration was obtained in this manner.

Following pretraining, the animals were given three days training in the Skinner box. On each of these days the rat was left in the box until 30

rewarded responses had occurred. Extinction of the bar pressing habit was started the day following the last day of the reward training. Extinction of the habit was accomplished by placing the animals in the Skinner box for a 20 min. period each day until a criterion of five or less responses per 20 min. period was reached. During extinction trials no reward was given for bar pressing responses. A record was kept of the responses per period and of the number of periods required for extinction.

A single unit T maze (previously described by Meier & Bunch, 1950) was used to measure habit reversal with Group II of the experiment. This maze was a tank, the stem of which measured 42 in. and the arm 36 in. The maze was 15 in. deep, 8 in. wide in the stem, and 6 in. wide in the arm. The ends of the arms were extended forward 6 in. parallel with the stem, making the ladder by which the animals were permitted to escape invisible from the choice point. Water was maintained at a depth of 9 in. at a temperature of approximately 70° F.

Habit reversal training in the single T maze was started for Group II at 120 days of age. All animals were required through massed practice to learn a simple left turning habit to a criterion of 10 successive errorless trials. Two days following original learning, the animals were required to reverse the left turning habit to a right turning habit. Again learning was by massed practice to a criterion of 10 errorless trials.

Results

Table 1 gives the means and standard deviations for trials and errors for the control and one-half hour anoxic groups for both original learning and relearning in the 14 unit maze. As indicated in the table, none of the mean differences was statistically significant.

TABLE 1

COMPARISON OF NUMBERS OF TRIALS AND ERRORS FOR INITIAL LEARNING AND RELEARNING OF 14 UNIT MAZE BY CONTROL AND ONE-HALF HOUR ANOXIC GROUPS

	Control (N = 54)	SD	Anoxic (N = 57)	SD	<i>t</i>	<i>p</i>
	Mean		Mean			
Initial Learning	22.89	14.86	22.30	15.24	.20	> .05
Trials:						
Relearning	6.81	4.94	7.42	6.99	.25	> .05
Initial Learning	173.93	125.36	154.98	104.16	.86	> .05
Errors:						
Relearning	25.69	25.66	25.13	23.62	.12	> .05

TABLE 2

COMPARISON OF NUMBERS OF RESPONSES AND DAYS NECESSARY FOR EXTINCTION OF A BAR PRESSING HABIT BY CONTROL AND ONE-HALF HOUR ANOXIC GROUPS

	Control (<i>N</i> = 15)	<i>SD</i>	Anoxic (<i>N</i> = 19)	<i>SD</i>	<i>t</i>	<i>p</i>
	Mean		Mean			
Responses	84.66	34.84	97.21	51.88	.79	> .05
Days	4.20	1.47	4.47	1.93	.45	> .05

Table 2 compares the control and one-half hour anoxic groups for the number of responses and days necessary for extinction of the bar pressing habit in the Skinner box. The lack of significant differences for these variables is demonstrated by the small *t* ratios given in the table.

Table 3 gives the means and standard deviations for trials and errors for the control and one hour anoxic groups for both original learning and relearning in the 14 unit maze. Examination of the *t* ratios reported in this table reveals that controls took fewer trials ($p < .01$) than anoxics on both original learning and relearning. Controls also made fewer errors for both sessions ($p < .05$) than did the group with one hour oxygen deprivation.

In Table 4 are found the numbers of trials and errors received by the control and one hour anoxic groups in learning a simple left turning response and then reversing it after a two day period. No significant dif-

ferences existed between the groups for trials or errors during position learning. Significant differences, favoring the control group, did exist for both trials ($p < .05$) and errors ($p < .01$) when the response was reversed.

A comparison of variances of controls and anoxics of Groups I and II for all variables measured is presented in Table 5. For Group I, significant variance differences occurred only in the case of relearning trials for the Stone maze. All variances differed significantly for Group II except the variances for errors in position learning.

Discussion

The results of this study indicate that deprivation of oxygen for a 30 min. period following birth does not affect later ability of rats to learn and relearn a Stone type maze or to retain a bar pressing habit. Deprivation of oxygen for a 60 min. period

TABLE 3

COMPARISON OF NUMBERS OF TRIALS AND ERRORS FOR INITIAL LEARNING AND RELEARNING OF 14 UNIT MAZE BY CONTROL AND ONE HOUR ANOXIC GROUPS

	Control (<i>N</i> = 23)	<i>SD</i>	Anoxic (<i>N</i> = 24)	<i>SD</i>	<i>t</i>	<i>p</i>
	Mean		Mean			
Original Learning	20.00	8.96	30.50	13.50	3.09	< .01
Trials:						
Relearning	5.56	3.77	10.46	7.64	2.74	< .01
Original Learning	149.04	63.48	239.11	160.32	2.50	< .05
Errors:						
Relearning	14.83	10.72	40.96	47.17	2.29	< .05

TABLE 4

COMPARISON OF NUMBERS OF TRIALS AND ERRORS FOR INITIAL LEARNING AND REVERSAL OF A POSITION HABIT BY CONTROL AND ONE HOUR ANOXIC GROUPS

	Control (<i>N</i> = 19)	<i>SD</i>	Anoxic (<i>N</i> = 21)	<i>SD</i>	<i>t</i>	<i>p</i>
	Mean		Mean			
Position Learning	12.68	2.18	13.62	5.30	.72	>.05
Trials:						
Position Reversal	11.95	1.10	14.52	4.28	2.60	<.05
Position Learning	1.47	1.04	1.33	1.25	.38	>.05
Errors:						
Position Reversal	3.95	1.82	7.95	3.80	4.21	<.01

following birth does affect the later ability of rats to learn and relearn a Stone type maze and to reverse a position habit previously learned. The meaning of the results may best be considered when compared with results of other recent studies.

Windle and Becker (1943) and Becker and Donnell (1952) found differences in the learning abilities of guinea pigs after subjecting halves of litters to severe oxygen deprivation by cutting off intrauterine circulation. Armitage (1952) reported that administration of varying doses of drugs to female rats on the day prior to parturition had a detrimental effect on the later learning ability of the young. Scheidler (1953) found that the offspring of female rats who had been exposed to a simulated altitude of 30,000 ft. for 2 hrs. just prior to birth learned less well than the offspring of normal mothers. Significant differences between anoxics (one-half hour at 3% equivalent oxygen immediately following birth) and controls in ability to reverse previously learned position habits are reported by Meier and Bunch (1950).

In contrast to these positive findings are the negative findings of two studies. Youngdahl (1948) deprived halves of litters of oxygen within 6 hrs. of birth. Deprivation was accomplished by nitrogen dilution of the atmosphere of a chamber to 5% equivalent oxygen for a 25-28 min. period. No differences were found in learning between anoxics and controls at 60 days of age. No differences were found by Hurder

and Sanders (1953) when they compared rats which had been exposed to a simulated altitude of 34,000 ft. for 34 min. at the age of 1 hr., rats exposed to 30,000 ft. for 3 hrs. at the age of 1 hr., and normal animals.

The authors believe that the deleterious effects of anoxia at birth have been tentatively established. They believe, further, that perhaps two factors are responsible for the varying results of Groups I and II in the present study and the positive and negative results of the studies described above. These factors are (a) the maturity of the experimental animals at birth and (b) the amount of deprivation received by the experimental groups.

In the studies (Armitage, 1952) in which guinea pigs were used as subjects (Ss) these factors acting in combination are responsible for the positive results. The guinea pig is very mature at birth with a consequent low resistance to anoxia (Fazekas et al., 1941). Very severe deprivations (almost complete denial of oxygen; in some cases oxygen or gas mixtures were required to revive the experimental animals) were used in these studies. Its severity is attested by the high mortality rate (36%) in the experimental groups. Therefore, low resistance combined with severe deprivation produce the consistent positive findings reported.

It is in the studies of rats that inconsistent findings are reported. These animals being very immature at birth can be expected to have a high degree of resistance

to anoxia (Fazekas et al.; 1941). Youngdahl (1948), Meier and Bunch (1950), and the experimenters in this study (Group I) used about the same degree of deprivation (approximately 3% equivalent oxygen, or 46,000 ft. for 30 min.). Hurder and Sanders (1952) subjected their animals to 34,000 ft. for 38 min. or to 30,000 ft. for 120 min. Mortalities for the studies reporting them are as follows: Meier and Bunch, overall mortality of the anoxic group exceed the controls by 2.1%; Hurder and Sanders, no losses from anoxic processing, two unspecified animals died; and Group I of the present study, 1% died while being rendered anoxic. Youngdahl does not report these data. With the exception of the limited findings of Meier and Bunch, no differences were found between the experimental and control groups in these studies.

Scheidler (1953) subjected pregnant rats to a simulated altitude of 30,000 ft. for 2 hrs. Armitage (1952) subjected pregnant rats to varying doses of drugs on the day prior to the birth of the young. Mortality rates in these studies were: Scheidler, 39% of the animals died during the deprivation period; and Armitage, 31% overall mortality in the anoxic litters as compared to 16% for the controls. Both of these studies showed positive results.

Consideration of the above findings has led the authors to make the following formulation which is believed to be of heuristic value:

In any large population it is likely that the ability to resist deprivation is normally distributed. Some few individuals will be readily susceptible, some few will be highly resistant, and the majority which lie be-

TABLE 5

COMPARISON OF VARIANCES FOR CONTROLS AND ANOXICS OF GROUPS I AND II FOR ALL VARIABLES

	Control Var.	Anoxic Var.	F Ratio	p
Group I				
Stone Maze				
Trials:				
Initial Learning	220.82	232.26	1.05	>.05
Relearning	24.40	48.86	2.00	<.01
Errors:				
Initial Learning	15715.13	10849.31	1.45	>.05
Relearning	658.44	557.91	1.18	>.05
df	53	56		
Skinner Box				
Responses to extinction	1213.83	2691.53	2.22	>.05
Days to extinction	2.16	3.72	1.72	>.05
df	14	18		
Group II				
Stone Maze				
Trials:				
Initial Learning	80.28	182.25	2.27	<.05
Relearning	14.21	58.37	4.11	<.01
Errors:				
Initial Learning	4029.71	25702.50	6.38	<.01
Relearning	114.92	2225.01	19.36	<.01
df	22	23		
Single T Maze				
Trials:				
Position Learning	4.75	28.09	5.28	<.01
Reversal	1.21	18.32	15.14	<.01
Errors:				
Position Learning	1.08	1.56	1.44	>.05
Reversal	3.31	14.44	4.36	<.01
df	18	20		

tween these extremes will be grouped around some mean resistance value. It is possible to divide the total area of this normal curve into many small areas so that the percentage of cases falling in each area is known. The anoxic tolerances for individuals in each of these small areas can be empirically determined by steadily increasing the amount of deprivation and noting mortalities. It is important to consider what happens to individuals in the small areas adjacent to that area in which fatal deprivation occurred. This is illustrated in Figure 1. Individuals to the right of this small area have all succumbed earlier because their resistance was less. Individuals to the left have been affected to varying extents. A graph can be made giving the proportion of fatalities in the population for each successive increment of deprivation. This assumes a linear relationship between deprivation and proportion of fatalities, although other predictable relationships could be substituted.

It has already been stated (Gomez & Pike, 1909; Grenell, 1946) that a gradient of resistance to anoxia exists; higher mental centers are more susceptible than lower centers. This would imply that a gradient of effect for a given level of deprivation occurs extending in the direction of high re-

sistance. A given deprivation would produce a proportion of fatalities. Individuals falling in that part of the curve adjacent to the area of fatality would be very heavily affected, at the point of death, with such affects diminishing as distance toward high resistance decreases.

The above formulation is important for statistical reasons. Let it be assumed that the normal curve for resistance to anoxia has a mean of zero and a sigma of one. Let it be assumed that the gradient of effect of deprivation extends for one sigma unit to the left of the area of fatality for any given deprivation. If a sample of individuals is submitted to oxygen deprivation to the extent that 5% are fatalities (Figure 1A), what is the effect on the rest of the population? In this case the distance from the edge of the fatal area to the mean of the distribution is 1.64 sigma units. If the gradient of effect extends one sigma unit towards the mean, 21.0% of the total sample has suffered some effect less than death. Let the case where the deprivation is such that fatalities have amounted to 25% of the sample (Figure 1B) be next considered. Here, the distance of the fatal area from the mean is .67 sigma units. If the gradient of effect extends for one sigma unit toward the mean, 37.8% of the sample has been affected to some degree.

In terms of learning scores, this would mean that only part of an experimental group could be expected to have a change in learning ability. The effect of a decrease in learning ability for part of an experimental group would result not only in lowering mean learning scores, but, also, in increasing variability for the experimental group as a whole. This is substantiated by the findings of the present study which are reported in Table 5. In the case of Group II, where the anoxics suffered considerable mortality, the variance for every learning variable save one is significantly greater for the experimentals.

It appears, then, that within certain limits and depending upon the gradient of effect the mortality rate in the experimental sample may be used as an index of the total

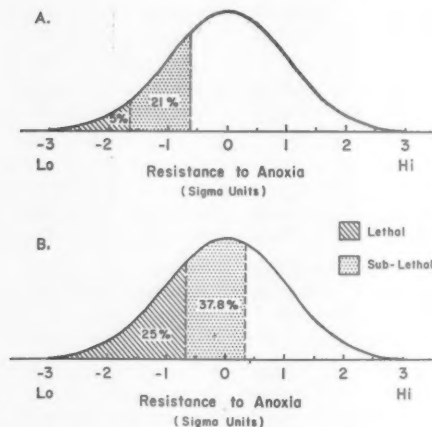


FIG. 1. Schematic representation of distribution of tolerance to oxygen deficiency and probabilities of subsequent alterations in behavior.

effect upon that sample. In studies such as those of Youngdahl, Hurder and Sanders, Meier and Bunch, and Group I of the present investigation, where very few or no animals died as a result of deprivation, minimal statistical differences existed between the control and experimental groups because the total effect on the experimental population was small. There probably were, however, individuals in the experimental groups who had been affected. The effect of the scores of these few individuals on the sample statistics was negligible, however.

To summarize the preceding paragraphs: in any species the effects of oxygen deprivation at birth depend upon the maturity of that species at birth and the amount of deprivation. More mature species require less deprivation to produce a given effect. In a sample of any species, the mortality rate of the experimental sample can be taken as an index of the total amount of affect in that sample.

Summary and Conclusions

Since the experimental literature appeared to be equivocal in specifying the role of natal anoxia in the etiology, we conducted this study in which both the severity of oxygen deprivation and type of criterion task were manipulated. One group of rats ($N=57$) was exposed to a simulated altitude of 46,000 ft. (2.91% equivalent oxygen) for 30 min., whereas a second group ($N=24$) was exposed to the same atmospheric conditions for 60 min. Fifty-four littermates served as controls for the first group; 23, for the second.

At the age of 65-72 days, both groups were given pretraining (five trials per day for 5 days) on a straight swimway.

Following the pretraining, the animals learned (to a criterion of one errorless trial) a 14 unit water maze of the Stone pattern. Thirty days after initial mastery, the *Ss* relearned the maze.

At the 150th day of age, the animals which had only 30 min. deprivation started learning an instrumental response. This consisted of: (a) 7 days adaptation to the food used in a Skinner box; (b) 3 days training in a "pseudo" Skinner box; and

(c) 3 days training in the Skinner box, per se. In the last, the time required for 30 rewarded responses was recorded on each of the days.

Extinction of the bar pressing response in the Skinner box was started after the last of the three days for acquisition. This continued until the animal made five, or less, responses in the daily 20 min. period.

The animals which had 60 min. deprivation learned a position habit in a single unit T maze when 120 days of age. Two days after learning a left turning habit to a criterion of 10 consecutive errorless responses, the *Ss* were required to reverse and learn a right turning habit to the same criterion.

From the data gathered in this fashion, we conclude that:

1. Thirty minutes of oxygen deprivation (2.91% equivalent oxygen) had no significant effect on the learning or retention of a multiple unit maze.

2. Further, this degree of oxygen deprivation had no discernible effect on the extinction of a bar pressing habit.

3. Sixty minutes of oxygen deprivation (2.91% equivalent oxygen) produced significant differences in the learning and retention of a multiple unit maze. The deprived animals were significantly inferior in the number of errors made and in the number of trials required to reach criterion on both the learning and relearning of the maze.

4. Although this more stringent degree of oxygen deprivation produced no demonstrable effect on the learning of a simple turning response, it did produce a deleterious effect on the reversal of that habit.

A comparison of these results with the results of similar studies, some of which reported positive findings, suggests that certain variables are of special importance in determining whether positive or negative results will be found. The differences in results among the various studies may be a function of the degree of deprivation used in the study and the physiological maturity of the experimental animals at birth. We have suggested that small amounts of deprivation, while seriously affecting some

few individuals in the population, would have little effect upon the statistics derived from a sample of that population. Possibly, the mortality rate in the sample may be validly used as an index of the total number of individuals affected in the sample by a specific amount of deprivation.

II. DELAYED EFFECTS OF NATAL ANOXIA ON BEHAVIOR AND ELECTROENCEPHALOGRAPHIC ACTIVITY

Two interests guided the course of the research discussed in this section: (a) the aspects of behavior affected by natal oxygen deficiency and (b) the relative development of electroencephalographic activity.

The aspects of behavior affected by natal oxygen deficiency. Experimental evidence discussed earlier in this monograph, as well as that published elsewhere (Fazekas et al., 1941) on this problem of natal anoxia, has shown that behavioral dysfunctions may occur, at least on unselected learning tasks. However, a few experimental studies (Becker & Donnell, 1952; Windle & Becker, 1943) and some clinical reports (Despert, 1946; Preston, 1945; Rosenfeld & Bradley, 1948) indicate that selected phases of the total behavior may be more severely impeded than others. Becker and Donnell (1952) report an increased incidence of stereotyped behavior among their experimental Ss. Despert (1946) has cited instances of extreme levels of anxiety; Preston (1945) and Rosenfeld and Bradley (1948) point out the difficulties of their clinical Ss in the mastery of tasks which require the use and manipulation of symbols and concepts (e.g., arithmetic).²

The S used in this research was the cat (a "higher" organism as compared with

those used in the other experimentation). We felt that stereotypy and symbol formation could be adequately demonstrated in this S. In line with the clinical evidence and that offered by Becker and Donnell, we predicted that those cats which had been deprived of oxygen at birth would evince a lesser variability of behavior and a poorer mastery of symbolic tasks than controls. We predicted poorer learning abilities on those tasks where stereotypy and symbol formation would probably not appear.

Relative development of electroencephalographic activity. Since the pattern of electrocortical activity immediately following anoxemia is well-known (Libet et al., 1941), but little is known regarding delayed or long-term changes, we initiated a program of electroencephalographic recordings on the Ss used in the behavior analysis. These recordings were scheduled to start at birth and continue at regular intervals for the duration of the experiment.

We predicted a change in the development of the response frequency: in the S with natal anoxemia, the development of the frequency of the cortical activity, normally from the slow, irregular waves of the infant to the moderate, regular waves of the adult, would be temporally delayed. At maturity the frequencies for the two groups of Ss were expected to be the same.

Subjects

In using cats as Ss, we capitalized upon the existing literature relevant to comparative behaviors on stereotypy, simple discrimination, multiple mazes, and problems evoking symbol formation. Further, by using this S we could utilize the considerable amount of material on neuroanatomy, electrocortical activity, and cortical function.

The cats were born of the breeding stock within the colony at Washington University. Although all the young were of known parentage within the colony, the strain was far from homogeneous; it was that which is commonly referred to as "alley cat" variety (a situation which presented some obvious difficulties, especially in the statistical analysis of the results).

Seventy-three litters (272 kittens) were used. These were born into the colony in the interim between March 1, 1950 and April 26, 1952. One hundred and forty-six of the 272 kittens were exposed to a deprivation of oxygen at birth. The

² Not all of the clinical studies of this topic have demonstrated positive relationships between neonatal anoxia and intellectual impairment. In a rare predictive study by Apgar, Girdany, McIntosh, and Taylor (1955), no significant correlations were found between blood oxygen content immediately after birth and performance on the Stanford-Binet test administered during early childhood. This failure to find a significant correlation is not easily understood in the light of other findings from the clinic and the laboratory.

remainder, 126 kittens, served as littermate controls.

Of those exposed to oxygen deprivation, 44 (30.1%) died, during or immediately after exposure. No single factor other than individual differences in tolerance could be denoted in the explanation of these fatalities. A certain percentage, by no means substantial, seemed to have had involvements related to nursing prior to the deprivation.

Behavioral data from the several tasks were collected on 26 of 272 kittens. Of these, 14 were experimental, i.e., they had been deprived of oxygen immediately after birth; the remaining 12 were the littermate controls. Differences in viability between the experimental and control groups did not appear once the deprivation and resuscitation were complete. Some fatalities appeared within the first week after birth (approximately 3% in both groups); these are best described as consequential to maturation level at birth and difficulties incurred during labor and birth processes rather than being causally related to the experimentally induced difficulties. In those litters where early postnatal deaths appeared, both experimental and control groups suffered. The remarkable decrease in the sizes of the groups (about 90%) as seen at birth and at the time of the completion of the experiment is sufficient attestation to the existence of impediments in the rearing of these animals (in large numbers, at least) through the first year of life.

Apparatus and Procedure

Deprivation

The infant cats were exposed to an oxygen deficiency for a single period within the first two hours of postnatal life. From work completed earlier by the authors and from preliminary investigations with infant cats, the severity of the oxygen deprivation was set at 2.91% equivalent oxygen (pressure of 4.16 in. Hg; simulated 46,000 ft.) for 30 min. An additional 15 min. were spent in attaining this condition and another 15 min. returning to normal atmospheric conditions.

The selection of these particular deprivation conditions was originally based on certain limitations in the equipment available when we were working with rats. Contrary to a suspicion engendered early in this study, these conditions did not afford a median lethal dose (LD_{50}) but something less (about LD_{50}), according to the tabulation at the end of the experimentation. (However, continuation with these deprivation conditions permit comparison of data compiled on rats under these conditions.)

In this study, deprivation was achieved through the use of a large, cylindrical decompression chamber (diameter, 54 in.; over-all length, 111 in.; see Figure 2 for details). The decompression was

effected by means of a large pump capable of creating a "near vacuum" within a matter of a few minutes. Normally, however, the chamber was evacuated (or filled) at a rate that would be comparable to an ascent (or descent) of 3,000 ft. per min. The conditions within the chamber were indicated on a mercury manometer and a conventional aircraft altimeter. The rate of decompression (and later, return to normal atmospheric pressures) was controlled through a valve sufficient to maintain an equilibrium with the vacuum pump at any level of decompression. This was indicated on an aircraft rate of climb indicator. All instruments were connected directly to the wall of the chamber at a point unaffected by drafts or eddies created by the change of air pressure.

Although the question has been raised repeatedly as to whether the decompression per se has some detrimental effect on the organism, it was felt that the well received work of Paul Bert in 1878 (Van Liere, 1942) furnishes conclusive evidence that it does not. (At least it has no effect within the limits imposed on this experiment.) For this reason the authors are convinced that studies in which decompression is the mode by which deprivation is produced should give results of the same order as in those experiments in which the oxygen is replaced by some inert gas, e.g., nitrogen and CO_2 together. The former method has the advantage of ease and readiness of control; the latter, the advantage of rapidity of effecting the conditions of deprivation.

After this single exposure to this degree of oxygen deprivation, the kittens were returned to their respective mothers and permitted to mature with their littermate controls.

Electroencephalography (EEG)

Shortly before, during, and shortly after the period of deprivation electroencephalographic

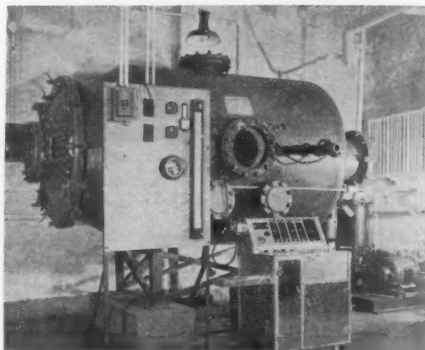


FIG. 2. Photograph of decompression chamber with two-channel electroencephalograph as used for recording during neonatal deprivation.

(EEG) recordings were made on the newly born kittens. As it was impractical to record continuously throughout this period (approximately 1½ hrs.) samples were made of 2 min. duration for each 10 min. of this entire period.

Follow-up recordings, of about 3 min. duration each, were made the day following the exposure (along with the littermate controls and the other experimental siblings that may not have had recordings made at the time of exposure) and at subsequent monthly intervals through the fourth month. Recordings were again made at the sixth month and at the end of the experiment. At this time they were at least 10 months old.

The monopolar recording technique was used. Leads were placed on the frontal lobes immediately posterior to the orbital ridge and lateral to the median line; on the occipital lobes posterior to an imaginary line extending from the trailing edge of one ear to that of the other, but lateral to the median line; and on both ears (the right ear served as a reference, the left as a ground); six electrodes were used in all (Figure 3). Due to difficulty in the continued placement of the disc electrodes through the movements of the *S*, needle electrodes were employed. (Twenty-seven gauge hypodermic needles were cut $\frac{1}{8}$ in. from the base of the stem. The lead wires were soldered to the flat surface of the base remaining.) EEGs were made on a Grass portable two channel recorder (model IIID). For the fourth and sixth month and final EEG recordings, pentobarbital was used to immobilize the animals (dosage: 30 mg./kg. body weight, administered intraperitoneally). Head holders and other restraining devices effectively stabilized the animals for the earlier recordings.³

The EEG recordings served as invaluable indices of the vitality of the exposed animals. They correlated well with the overt behavior at this time. When the animals showed a decrease in activity (as the lowest oxygen concentrations were reached), these records showed decrease in frequency and amplitude of the wave forms. Through the 30 min. at this level, both continued to decrease until an extremely low level of electrical activity was attained. In some *Ss*, the depression obliterated all recordable electrical activity except at those infrequent times when the infants gasped for air (much less than once each minute).

The overt behavior of the organisms and the concomitant electrical activity evidence the fact

³ D-Tubocurarine, curare, although superior in theory, had been found to have widely divergent effects on the animals, especially the young kittens. From the self-same dosage insufficient immobilization or fatality resulted. Pentobarbital and other barbiturates were not employed at this time because of a possible synergistic effect to the anoxemia (Himwich, 1951; Snyder, 1949).

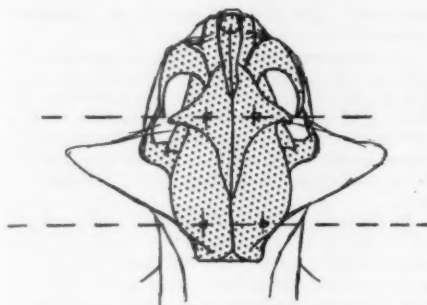


FIG. 3. Representation of head of a kitten to illustrate the placement of electrodes for electroencephalography with regard to processes on the skull. In addition to the four sites indicated (+), electrodes were also placed on either ear for reference and grounding purposes.

that the effective period of deprivation extended beyond the prescribed 30 min. at the low pressure of 66 mm. Hg (2.91% equivalent oxygen). Even and regular breathing was not resumed until some time after the animals were returned to the litter (usually at least an hour).

Behavioral Tests

Upon reaching the age of six months, the kittens were started on a battery of behavioral tests (largely learning) which were, in the order of presentation: (a) Guthrie puzzle box; (b) single unit T maze adapted for black-white discrimination; (c) revision of the Hunter Temporal Maze; and (d) feline adaptation of the Wisconsin General Test Apparatus. The presentation of all the learning problems had certain aspects in common: The animals were run in blocks of 20 trials each day. At this time the cats were 20–22 hrs. hungry. The reward in all cases consisted of pieces of kidney (about $\frac{1}{2}$ in.). Individual animals were trained on the apparatus in small groups in which both the experimental and control *Ss* were members.

The various learning tasks will now be described individually:

Guthrie puzzle box. The physical nature of this piece of apparatus was much as it is described in the monograph, *Cats in a puzzle box* by E. R. Guthrie and G. P. Horton (1946). The release mechanism was very similar to that depicted as the "B" variation in this monograph. It consisted of a pole 13 in. in length and $\frac{1}{4}$ in. in diameter mounted on a hemisphere of wood 4 in. in diameter. Any horizontal movement (at least $\frac{1}{4}$ in. at the top of the pole) was sufficient to make electrical contact and activate a solenoid to open the door permitting escape and access to the food (see

Figure 4). The inside dimensions of the retaining box (the main part of the puzzle box) were: 30 in. wide, 24 in. deep (the floor was an extended half circle with a radius of 12 in.), and 34½ in. high. Above the glass face of the box was a blackboard 6½ in. high, situated to indicate the date, the number of the trial, and the number of the S.

Within the circuit for the escape mechanism, contacts were made to operate a camera (35 mm.) which "caught" the cat as it manipulated the pole to escape from the apparatus.

The animal was timed from the moment of entry into the retaining box to the time the solenoid was activated and the door opened.

Little preliminary training was required. Five trials were given to familiarize the animal with the goal: a dish of food on the platform in front of the escape box. Ten more trials were given to goal-orient the animal by permitting him to run from the starting compartment, through the retaining box, and out the escape door to the food. (The pole was not in position.)

Five different positions were available for placement of the pole: middle, left front, left rear, right front, and right rear. The first 50 trials were with the pole in the middle position; the next 20 with the pole in the left rear position; the last 10 with the pole in the right front position. Thus, all Ss had 80 experimental trials.

The reason for the choice of this particular problem stems largely from the article by Windle and Becker (1943) in which the authors alluded to the stereotypy of the anoxic animals in the simple problem used to test their learning ability. Guthrie and Horton claim that by analysis of the photographs taken at the moment of operation of the escape mechanism they were able to infer stereotyped behavior to their Ss. (By stereotyped behavior Guthrie and Horton meant similarity of

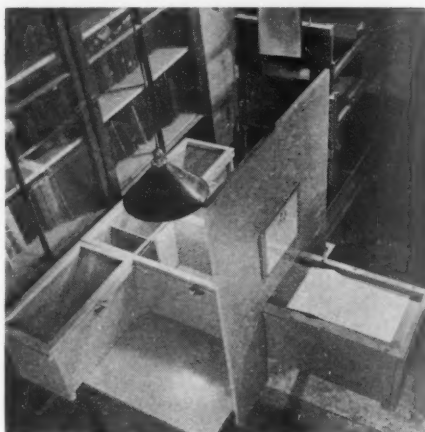


FIG. 5. Top-side view of T maze discrimination apparatus.

bodily position and contact in the act of manipulating the pole.) These two statements were integrated in the construction and usage of the apparatus. Both the experimental and the control Ss should, according to Guthrie and Horton, show this form of stereotyped behavior. However, if Windle and Becker's statement is valid, at least for cats, the natively asphyxiated group should show this to a greater extent than the control group.

As a further check on this issue, the various positions of the pole were utilized as indicated above. That group which possessed the greater degree of stereotypy should operate the escape mechanism in a new, unfamiliar position less readily than formerly.

Discrimination problem: Single unit T maze. The apparatus used to test the ability of the cats to discriminate brightness was of conventional design: a simple, enclosed, single unit T maze. The stem of the T was 40 in. long; half of this length comprised the starting box, the other half the straightaway to the choice point. A vertically operated door divided the starting box from the stem. The arms were 24 in. in length. A partition located at the junction of the arms with the stem separated the arms, one from the other. At this point a brass rod was fitted across the stem from which the swinging doors (one-way) were suspended (see Figure 5).

The maze was 10 in. wide in the stem and 8 in. wide in the arms; it was 10 in. deep throughout.

Twenty-five preliminary trials sufficed: 5 to instruct the S in running forward from the starting box; 20 to goal-orient him to the left and right goal boxes (swinging doors were not in position at this time).

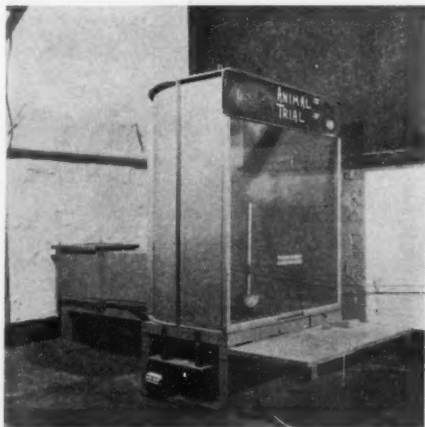


FIG. 4. Front-side view of Guthrie puzzle box.

Subsequent to these 25 trials solid gray doors were placed at the choice point, partly to acquaint the cat with the operation of the doors, partly to teach him left and right position habits (depending on his preference, although instructed that reward was to be found in both goal boxes). After making this choice as to the preferred side (five consecutive trials to one side), the *S* was trained to go to the opposite box in accordance with a noncorrective technique. Here the criterion was 10 consecutive trials to the opposite goal.

Upon mastery of these two position habits the *S* was confronted with the task of learning a brightness discrimination habit. In this instance the gray doors were removed and solid black and white ones substituted. The black door was positive for all *Ss*; its position varied randomly from left to right according to a prepared list. The criterion of mastery was 10 consecutive perfect trials. A maximum was arbitrarily set at 300 trials.

Double alternation problem: Hunter temporal maze. The piece of apparatus used in this problem was of an original design suggested by F. H. Palmer (1950). Palmer conjectured that one of the factors that created difficulty in learning Hunter's maze was the number of 90° turns that had to be made which were not relevant to the crucial turns at the choice point. His results on a revision of the maze bore out this contention.

The version of the maze used in this study carried Palmer's plan to the extreme. In Palmer's design the outside corners were rounded leaving still the corners at the intersection into the stem leading to the choice point. The current revision consisted of a single T with 10 in. arms and stems.

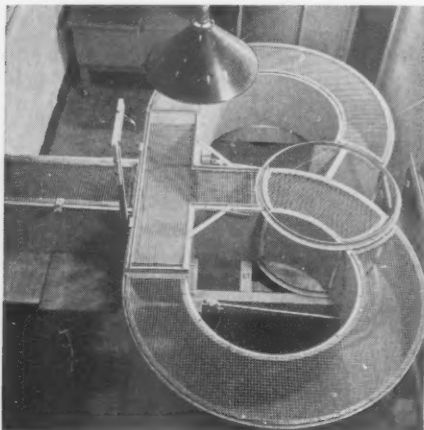


Fig. 6. Top-side view of temporal maze. The starting box is below small segment of arc in the right center of the picture; the goal box is at the left center.

Circular segments (10 in. inside radius) joined the ends of the arms of the T to the stem. Where the two circular segments approached the stem of the T, an arc of the segments was movable so that it could complete the left or the right path (see Figure 6). The passages were 8 in. wide and 10 in. deep throughout.

The starting compartment was below the moveable arc that completed the left and right paths. This was maneuvered into position by depressing a lever, thus raising the arc and the starting compartment (see Figure 7).

The goal box was an extension of the stem of the simple T beyond the choice point.

One-way swinging doors were placed at the ends of the arms of the T and at the start of the stem. These the *Ss* operated. A door was also placed at the goal box, concealing it from the animal as he entered the choice point. This the experimenter operated.

The particular double alternation problem was left-left-right-right (LLRR). Reward followed the completion of the entire problem.

At least 90 preliminary trials were required before the *Ss* could attempt to learn the pattern. Of these, 20 were spent orienting the animals toward the goal; the animal ran from the starting compartment directly to the goal box with all doors open. The next 10 were the same except that now the swinging door into the stem of the T was closed.

In trials 31-65 the animal learned to make a single choice at the intersection of the T. (The door to the goal box was now closed initially.) The "correctness" of the response, whether left or right,

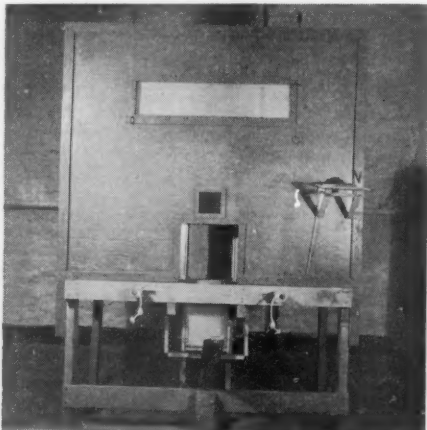


Fig. 7. Front view of temporal maze. The starting box is in the lower center of picture. Depression of treadle raises this box into starting position.

varied randomly according to a prepared list. During the subsequent 20 trials, and in the 5 following them, the *S* learned to put two and three, respectively, of these choices together into a single trial. Again, the correctness of the responses varied in a random fashion. Putting four such choices into a single trial became the actual problem that was to be learned.

The criterion chosen for mastery of the problem was three consecutive errorless trials.⁴ Along with the number of trials required to reach this level of performance, the errors made on each of the four legs of the task were recorded.

The choice of the double alternation problem is attributable to the role of symbolic behavior that has been reported for it, even though this reputation has not gone unchallenged (Seward, 1948). It was conceived to serve a position in this battery of tests analogous to those of symbolic functioning used in some of the clinical studies.

Discrimination problem: General test apparatus. The apparatus used here was an adaptation of the general test apparatus designed by H. F. Harlow for his studies of perceptual learning in monkeys (Harlow, 1951). The over-all base dimensions were 20 by 40 in. Of this, the full width (20 in.) and 18½ in. of the length comprised the restraining box from which the cat manipulated the discriminanda. The height of this box was 16 in. A vertically operating door separated the box from the remainder of the apparatus. At this point, inside the vertically operating door, two pieces of plate glass were so arranged as to form a horizontal slit (1½ in. high) at the level of the tray (3 in.) upon which the discriminanda were placed. This tray extended the full width of the apparatus, being of itself 10 in. wide. Two 2 in. depressions (6 in. between centers) in this tray served as receptacles for the food reward.

A second vertically operating door separated the tray from the experimenter, concealing him from the *S*.

A fluorescent light mounted on the door in front of the *S* illuminated the discriminanda and the tray (see Figure 8).

The cat was given some 20 trials to practice using the paws to secure the food from the tray. In an additional 10 trials he was instructed in the movement of the discriminanda and subsequent exposure of the food.

Thereafter the animal was confronted with the problem of selecting the black object (3½ in. squares were used) over a white object to procure the reward. A corrective type training procedure was used; the *S* persisted until a correct choice was made; this constituted a single trial. As with the previous discrimination task (single unit T

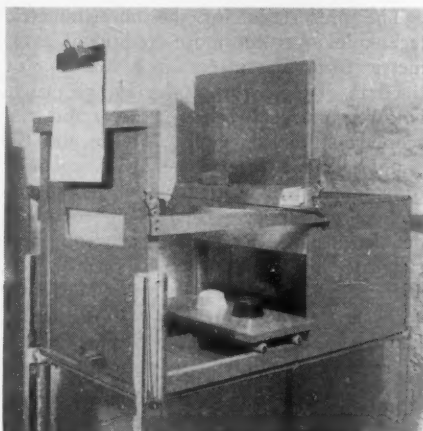


FIG. 8. Front side view of adaptation of Wisconsin General Test Apparatus.

maze), 10 consecutive errorless trials constituted the criterion of mastery.

The use of a second discrimination problem was occasioned by several observations of the single unit T maze. Watching the experimental animals in their fixated behavior in this maze, the experimenters felt that part of this behavior could be explained by a differential motivating factor. Possibly the experimental animals were not as highly motivated for food as were their littermate controls. On every trial in the maze the animals were rewarded at least to the extent of being picked up and handled by the experimenter. It was conceivable that this was sufficient to drive the animal forward in the maze, but being the same for both the "right" and "wrong" choices did not reinforce the animal in the learning of the black-white discrimination.

Such an explanation could also be utilized in those instances where the animal did not learn the discrimination but still did not fixate (this included both experimental and control *Ss*).

Results

Statistical Analysis

The significance of differences between the two groups of animals in their performances on the behavioral tests was determined by nonparametric procedures. To compare central tendencies, the Mann-Whitney *U* Test (Moses, 1952) was used; for contingencies, Fisher's (1946) exact treatment of 2 by 2 tables was used.

⁴ The criterion chosen for mastery of the problem was three consecutive errorless trials. If interaction is absent, the probability that this could occur simply by chance is 1:4096.

The preference for the nonparametric techniques over the more traditional parametric forms lies in certain restrictions of the data. Time scores on the Guthrie puzzle box were markedly skewed. Error and trial scores on the single unit T maze and the temporal maze were such that homogeneity of variance was lacking. Admittedly, corrections might have been made for these deficiencies by way of such procedures as suggested by Cochran and Cox (Edwards, 1950; Johnson, 1949) and McNemar (1949). In any event, even with the loss of efficiency inherent in aspects of this analysis of results, the statistics used seemed to us to be the best choice.

As stipulated by the nature of the hypotheses examined here, one-tailed tests of probability prevailed (Jones, 1952).

Subjects

In the final outcome, the number of Ss (N) varied from test to test within the behavioral analysis. Three Ss died after completing part of the test battery. Some Ss were withdrawn from individual tests because of a failure to respond. (See the final paragraphs in this section.) Consequently, N varied between 21 and 24.

Behavioral Results

Guthrie puzzle box. Two scoring techniques were employed with this apparatus. The first utilized the latencies of response for each trial. Comparisons were made be-

tween the two groups as to the mean latency for successive blocks of 10 trials. The trials on three tasks were analyzed: (a) the release mechanism in the middle of the restraining cage; (b) in the left rear position; and (c) in the right front position. In the second technique, the repetitiveness of responses for each animal was determined. The tabulation was made from the photographs taken at the moment of activation of the release mechanism. Only the first 50 trials were considered. (The feeling was maintained that the subsequent 30 trials would show little in the way of stereotypy of response: the rationale for incorporating this instrument in the battery of tests. Reviews of the photographs sustained this belief.)

Significant differences were revealed in the later blocks of trials on the first task and in the first 50 trials, collectively. The performances on the last task (Trials 71-80) also demonstrated statistically significant differences. In every case of significant difference, the latencies for the controls were shorter than for the experimental animals. (Only in one of the nine comparisons made was the median latency for the experimental group shorter than the median for the control group.)

The study of the photographic records proved unrewarding. In this analysis each of the photographs (taken at the time of activation of the release mechanism) was considered and rated on several dimensions of response. To compensate for any dis-

TABLE 6
GUTHRIE PUZZLE BOX
Time Scores

Trials:	1-50		1-10		11-20		21-30		31-40		41-50		51-60		61-70		71-80	
Group:	C*	X**	C*	X**	C*	X**	C*	X**	C*	X**	C*	X**	C*	X**	C*	X**	C*	X**
Median	7.4	10.3	15.7	23.6	8.8	7.9	5.1	9.3	2.9	7.3	2.2	6.2	4.1	7.8	5.4	8.3	6.3	20.5
Mean	9.2	41.6	35.0	116.5	9.6	36.5	6.0	15.6	6.5	19.4	5.0	11.8	7.4	13.8	27.2	15.4	6.3	28.6
SD	3.9	56.8	52.8	141.3	4.3	82.8	3.0	15.5	6.3	26.2	4.7	12.1	5.6	15.8	3.7	15.9	1.2	31.8
$p(U)$	<.05				<.05		<.06		<.05		<.05						<.05	
$p(Run)$																	<.025	

* $N = 24$.

** N , the number of Ss used, varies with each problem. This variance was due to the withdrawals necessitated in each instance. Note.—Only those probabilities approaching the 1%-5% levels of significance are indicated in this table.

TABLE 7
GUTHRIE PUZZLE BOX
(Percent of responses)

Group:	Response/Total		Part/Total		Side/Total		2 X /Total	
	C	X	C	X	C	X	C	X
Median	.29	.32	.49	.49	.64	.63	.28	.35
Mean	.33	.36	.52	.52	.64	.67	.29	.34
SD	.15	.18	.12	.19	.11	.20	.12	.16

Note.—Probability (*U*)—all greater than .05; *N* = 24.

crepancies in the number of trials available to this analysis, these tabulations were converted to percentages and the following indices were derived: the proportionate frequency of the dominant localized response (the side and part of the body contacting the pole), the frequency of a dominant response not considering the side of the body involved, the frequency of several responses involving one side only, and the frequency of immediate duplications of a localized response. No significant differences were revealed.

Single unit T maze. For the three tasks on this apparatus the scoring technique was the same: the number of trials to reach criterion. For the first position habit, the criterion was 5 consecutive responses to one side; for the second position habit and the discrimination task, the criterion was 10 consecutive errorless performances.

Analysis of the performances (in terms of trials to criterion) on the two position tasks by the Mann-Whitney *U* test failed to show significant differences between the two groups.

As there was an upper limit to the number of trials given to each *S*, enforcing an artificial "cut-off" on the distribution of scores, and as there was elicited performance not conducive to learning the discriminatory response, the chi square

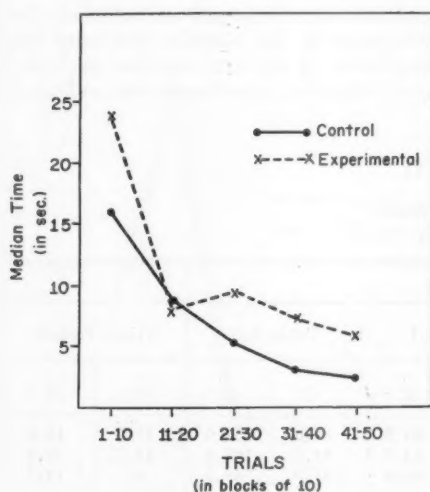


FIG. 9. Comparison of performance, in time scores, of the two groups over the first 50 trials on the Guthrie puzzle box.

TABLE 8
SINGLE UNIT T MAZE
(Trials)

Group:	Task		Task	
	1st Position		2nd Position	
	C	X	C	X
Median	20.5	30.0	28.0	28.0
Mean	20.8	40.8	26.2	31.1
SD	6.7	28.3	10.3	11.5
Probability (<i>U</i>)	> .05		> .05	

Note.—*N* = 23.

TABLE 9
FREQUENCIES OF SUCCESSFUL PERFORMANCE ON
BLACK-WHITE DISCRIMINATION PROBLEM

	Learn	Not Learn	Total	<i>p</i>
Control	8	2	10	.034
Experimental	4	8	12	
Total	12	10	22	

technique was used with the data on the discrimination problem. Two separate analyses were made: (a) the frequencies of those who learned and those who did not learn were compared for the two groups, and the results are given in Table 9; (b) the frequencies of those who fixated (as defined earlier) and those who did not are given in Table 10.

TABLE 10
FREQUENCIES OF FIXATIONS OF BLACK-WHITE
DISCRIMINATION PROBLEM

	Fixate	Non- fixate	Total	<i>p</i>
Control	0	10	10	.030
Experimental	5	7	12	
Total	5	17	22	

TABLE 11
TEMPORAL MAZE
(Trials)

Group:	Score							
	Total Trials		Trials: 0-1		Trials: 1-3		Trials: Perfect	
	C	X	C	X	C	X	C	X
Median	182.0	220.5	97.0	65.5	82.0	159.0	15.0	18.0
Mean	178.0	232.7	86.0	64.7	93.6	167.8	15.7	21.9
SD	47.7	71.2	32.7	46.0	47.9	85.3	6.1	11.2
Probability	< .06		> .05		< .05		> .05	

Note.—*N* = 19.

As indicated by the probabilities given to the right of the paradigms, above, both analyses demonstrated significant differences beyond the .05 level. The frequencies of *Ss* who did not learn the discrimination and/or fixated were greater for the experimental group than for the control group.

Double alternation problem: Temporal maze. The number of trials necessary to reach the criterion of three consecutive errorless performances and the number of errors made in so doing were the scores used for this problem. The latter, the number of errors made, was recorded in terms of the errors made on each of the four "legs" on a single trial.

When the number of trials were compared, certain significant differences appeared. In terms of the total number of trials to reach the criterion of three consecutive errorless trials, the differences were significant between the .05 and .06 levels, in favor of the control group. The analysis was made with the Mann-Whitney *U* Test. This statistic did not demonstrate a significant difference in the comparison of the numbers of trials required to reach one errorless performance. The difference between the groups on the total number of trials seemed to be largely attributable to the differences in the behavior following the completion of the first errorless performance. Statistical significance was indicated

Gro
Me
Me
SD
Pro

bey
diff
M
as
Co
num
as
the

Medien Errors
1

Fr
score
ment

TABLE 12
TEMPORAL MAZE
(Errors)

Group:	Score									
	Total Errors		Errors 1		Errors 2		Errors 3		Errors 4	
	C	X	C	X	C	X	C	X	C	X
Median	294.0	333.5	69.0	67.5	205.0	227.5	3.0	9.5	4.0	4.5
Mean	274.3	350.8	66.3	64.0	204.7	251.8	6.0	9.3	5.1	5.5
SD	97.6	88.0	30.0	24.4	68.9	86.0	5.0	3.5	4.6	5.6

Probability—all were greater than .05; $N = 19$.

beyond the .05 level in the analysis of this difference.

No significant differences in performance, as measured in errors made, was found. Comparisons were made upon the total number of errors made in reaching criterion as well as upon the errors made on each of the four legs of the task.

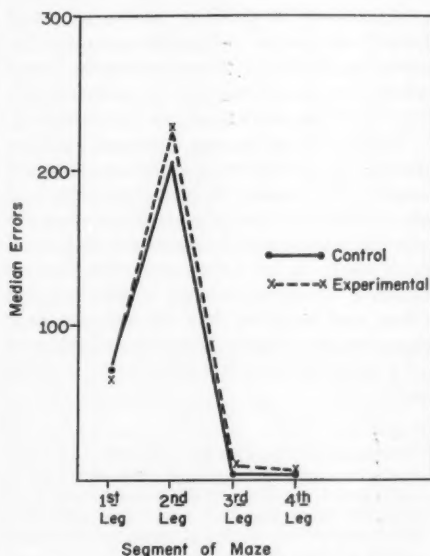


FIG. 10. Comparison of performances, in error scores, of the two groups on each of the four segments ("legs") of the double alternation task.

Discrimination problem: General test apparatus. Although used to corroborate the other test for brightness discrimination (above), the general test apparatus denoted no significant differences between the two groups. There were discrepancies between the medians, in favor of the experimental group, but these lacked statistical confirmation (Mann-Whitney U Test).

The results on this apparatus bore low correlation with the similar data from the single unit T maze.⁵ All S s learned the problem; this included even those who fixated on the earlier task. Some of the most rapid learners were those who failed to learn the correct solution of the discrimination.

In addition to these data compiled from the behavior tests, other impressions of group peculiarities were formed. It was noted, for example, that the experimental animals were more irritable in the learning situations. Should some untoward event occur, which it inevitably did for both groups, it was more likely that these animals would "balk."⁶ They would refuse to con-

⁵ The coefficient of correlation (Kendall's tau) for biserial rank order (1948) with these two sets of data was +.24. On a test of the null hypothesis for the reliability of this correlation, $p = .23$.

⁶ These untoward events consisted of unexpected noise within or without the apparatus, unexpected resistance of some movable part, e.g., doors in the temporal maze, or some unprescribed physical restraint or injury.

TABLE 13
MULTIPLE CHOICE APPARATUS
(Trials)

	Control	Experimental
Median	47.50	44.0
Mean	57.80	45.4
SD	27.9	15.4

Probability > .05

Note.— $N = 24$.

tinue operation of the problem impelling added encouragement or preliminary type training (even though they were in the midst of the actual learning trials).⁷

Further, the impression prevailed that the experimental animals were less active and responsive in their home cages. The animals tended to remain immobile in a corner of the cage seemingly unmindful of the activity of the other animals (their litter-mates) or of the proximity of the experimenter and others concerned with the colony.

It was apparent, however, that the single period of oxygen deprivation had little or no effect on normal sexual functioning. Whether it had some effect on the maturation of these functions could not be inferred from the observations. Certainly it did not alter the existence of breeding and maternal behavior.

Electroencephalographic Results

The electroencephalographic data were uniformly negative, i.e., no consistent differences could be found between the two groups at any stage of development. Because of the wide variability of the electrical activity between recordings and within a single recording for a given *S*, a statistical analysis of the records on immature *S*s was not feasible. Within the accuracy of the measures, the data on the adults were all but identical, and further statistical analysis

was judged unnecessary. In a blind run through a random series of records from both groups, an accurate selection of "controls" and "experimentals" could not be made.⁸ (For further data on the electroencephalographic recordings, see Appendix A.)

Discussion

These data indicate that the nature of stereotyped behavior described by Guthrie and Horton (1946) and that described by Windle, Becker, and Donnell (1943, 1952) are not necessarily related, certainly not identical. According to Guthrie and Horton, if one group of *S*s were more stereotyped than the other, then the one would show a greater proportion of related behavioral patterns in the operation of the escape mechanism. It is true that in this study both groups did show the repetitive type of behavior with which Guthrie and Horton concerned themselves; typically, both groups demonstrated this in like amounts.⁹

It follows also, although not necessarily so, that the stereotyped group would show a shorter, or at the very worst, an equal latency of response. The data are quite the contrary. Generally, the experimental group which was hypothetically the more stereotyped was the more sluggish in responding.

The type of stereotypy discussed by Windle and his coworkers was illustrated in the single unit T maze. A given percentage of the *S*s failed to learn the discrimination but selected one arm of the maze and responded persistently to it. Of course, this was an adaptive response, but one demanding less effort and attaining less reward. As only experimental animals demonstrated this type of adaptation, the first hypothesis is given substance.

⁸ It is entirely possible that, had there existed a satisfactory means of quantifying data which would depict variations in the voltage input, some conclusion other than "no differences" may have been made of the relative electroencephalographic activity of these two groups.

⁹ The series of duplicated responses were not as long, for any *S*, as Guthrie and Horton described with their *S*s.

⁷ In those instances where this additional acclimation and pretraining were ineffective, the animals were withdrawn from the particular problem.

Two variables, one related to each of these problems (puzzle box and T maze), may bear consideration for the understanding of the differences in these two groups of animals. In the Guthrie puzzle box, the ambiguity of the response-reward relationship is apparent. The control group learned the problem much more rapidly (cf. latencies on individual trials) than did the experimental. It is possible that similarity of successive responses is unimportant to the solution. In the single unit T maze, the delay in reward and the lack of secondary cues are apparent. Again, the control group was superior. Should these two variables be meaningful in the interpretation of the differences between these animals, stereotypy relates to diverse aspects of behavior (or, at least, has two diverse operational definitions).

The performance of the animals on the double alternation problem verifies the notion that the natively anoxic Ss are inferior in their ability to learn a complicated task involving symbol functioning. Although the groups were not significantly different in the rapidity of solution (i.e., in the number of trials required to make an errorless performance), nor in the pattern of learning the problem (i.e., which leg was learned first, second, etc.), they did differ in the ability to retain that solution and demonstrate that acquisition by consecutive errorless performances. The appearance of the first errorless trial may have been a chance phenomenon ($p = \frac{1}{18} = .0625$). The probability of three consecutive such trials ($p = 1/4096 = .0002$) makes untenable an explanation of these data on the basis of chance alone.

If this double alternation problem is adequate for an operational definition of symbolic behavior, then the data corroborate the observations of Preston (1945) and Rosenfeld and Bradley (1948): Individuals with a history of anoxia during infancy demonstrate a deficit in the ability to acquire and sustain the usage of symbols in their adaptive behavior.

Statistically, the results on the general test apparatus are such that the null hypothesis

cannot be rejected. The differences between the two groups of Ss were not so large (nor in the proper direction as specified by a one-tailed test of significance) as to be significant at the usual levels. Nevertheless, a sizable difference (medians, 8%; means, 21%) did exist suggesting that the experimental animals learned the discrimination more readily than did their controls. Whether further experimentation will confirm these differences or the null hypothesis will have little import on the over-all conclusion from these data on the discrimination and stereotypy (as defined in the single unit T maze): The experimental Ss adapted in a manner not wholly alike that of their controls. The failure to learn a brightness discrimination on the single unit T maze was not effected by an inability to detect differences in brightness discriminanda.

In their entirety the electroencephalographic data were disappointing. This conclusion stems not so much from their failure to confirm or reject one of the hypotheses as from the difficulty of instrumentation and analysis which they engendered. Unusual frequencies appeared in both groups to an equal extent. These were ostensibly consequential to difficulties in the placement of the electrodes (identity of position, in particular), in technical control (electrode resistance, movement of the skin, or presence of underlying muscle groups and sinuses), and in the inability to maintain a quiescent S without extensive reference to anesthetics or other drugs. For the younger Ss, the frequencies were widely variant within a single recording. When repeated recordings were made, this variance was increased. All in all the immature EEGs were remarkably unstable (as judged by the type of analysis used here).

With adult recordings, the frequencies were very much more regular. Frequencies and amplitudes found at one point in the recording were usually found throughout. Repeated recordings on a given S were extremely similar. Only at this time did the data appear reliable. At this time no differences between the groups were found—an expected result.

In general, the offhand, unquantified observations on the relative behaviors support a hypothesis which explains the origin of some, maybe all, of the tested behavior dysfunctions in terms of activity levels. This hypothesis states that for the development of normal, adaptive behavior (including functioning in contrivances of the psychologist's sort) a level of responsiveness must be maintained. Especially must this level of responsiveness be maintained during the early stages of development. Physiological insults, as oxygen deprivation, alter this responsiveness, but only temporarily. Cell changes may follow the insult; the cells may degenerate and die but are, shortly, removed and replaced (e.g., cortical neurones may be destroyed by the oxygen deprivation to be replaced, later, by glial cells; the usual failure to find specific, localized, permanent, cerebral histopathology gives empirical support to this hypothesis). Nevertheless, this transient effect of the insult may have far-reaching influences should the period of unresponsiveness be sufficiently long and occur at a critical point in the organism's growth. (Certainly, neither the length of the period nor the point of its occurrence, the critical point, can be specified at this time.)

Surveying the whole of the experimental design and the results gained, we feel that the material, as well as the problems reviewed by Royce (1950), have considerable methodological significance in experimentation of this nature. Using a well-formulated, standardized battery of tests with known intercorrelations denoting conceptualized factors (with operational significance), the experimenter could greatly facilitate the analysis of certain problems of a physiological and/or developmental character (e.g., natal anoxia, prenatal and natal irradiation, hypoglycemia, hypothermia, etc.). Experimental insults, as imposed upon the Ss in this study, may have a few general or a multitude of particular effects on the organism. To have such a test battery designate these effects and clarify their causal relationships with such variables as age of the S and length, frequency, and severity of the insult would alter the iso-

lated existence that such problems as these maintain in the field of physiological psychology, *in toto*. It is plausible that this methodology would extricate from these data such relevancies as would tend to instigate meaningful theorization across the demarcations of specific experimental problems and specific species of animals.

Summary and Conclusions

Behavioral measures were made on 26 cats to evaluate hypotheses concerning the effects of natal anoxemia on learning, stereotypy, symbol formation, and on the development of patterns of electroencephalographic activity. Data of both clinical and experimental origin were presented to indicate the definite possibility of marked effects of anoxemia at birth upon these forms of behavior.

Of these Ss, 14 were exposed to oxygen deprivation immediately after birth. By means of a decompression chamber the existing atmospheric conditions were reduced to 2.91% equivalent oxygen under which the animals were maintained for 30 min. EEGs were collected at this time and at monthly intervals thereafter. With the exception of the initial recording, concurrent recordings were made on the remaining 12 Ss, the littermate controls.

At the age of six months the behavior of the animals was observed on a battery of tests: Guthrie puzzle box, single unit T maze, temporal T maze, and a general test apparatus. The cats were to learn a simple brightness discrimination habit on the second and fourth tests and a double alternation habit on the third.

These conclusions were drawn from the observations:

1. The degree of oxygen deprivation incurred in this study altered the Ss' behavior in such a way as to significantly decrease the rapidity of response in the puzzle box.

2. These animals demonstrated an increased propensity towards poorly adapted responses. They tended to "stereotype" at certain discrimination tasks; rather than learn the proffered discrimination, they would learn a position response.

3. These animals did not manifest differences in ability to learn brightness discriminations in situations in which there was a close spatial and temporal proximity of response to discriminanda and reward.

4. They evinced differences in ability to cope with symbol formation, specifically, in their ability to sustain a symbolic solution after once the solution was gained.

5. They did not show differences in electrical activity (EEG) as seen at adulthood. (Difficulties in instrumentation and analysis prevent conclusion on the development of these patterns.)

III. THE EFFECTS OF PRENATAL ANOXIA ON LEARNING IN RATS

Although the cellular action of an oxygen deprivation prior to birth may be the same as similar deprivation later (Schreiber & Gates, 1938), the differences in the stages of development may be a significant factor circumscribing alternations in the organism's ultimate level of functioning. There is considerable evidence (Breslin, 1956; Ingalls, 1947a, 1947b) that insults at specified periods in the fetal life will effect changes in tissues which are developing maximally at that time. Some evidence (Meier & Menzel, 1955) has appeared indicating that such insults may lead to specific alternations in behavior as well.

In order to appreciate the significance of physiological insults upon the prenatal organism, such as oxygen deprivation, the significance and scope of the developmental activities in this period must be emphasized. This is the time in which occurs most of the growth and development of the organism—more than the sum total of such activities in the remaining vastly more lengthy span of its life. Most of the characteristics for physical and behavioral development are established during this short interval.

The study to be described here was designed to investigate the effects of prenatal oxygen deprivation and, further, to relate the nature of the effect to the age of the prenatate at the time of the deprivation. Specifically, the abilities of two groups of animals to learn and retain a simple and a

complicated maze pattern were measured. The first group, the experimental, was born to mothers which had been exposed to an oxygen deficiency via a decompression chamber. The second, the controls, was born to mothers of the same stock as the first but which were maintained under normal colony conditions for the entirety of the gestation period.

Part I

Subjects

A population of 80 female albino rats (Wistar strain) from the Buckeye Laboratories in Ohio was the source of the Ss observed in this study. Randomly, half were designated for the control and half for the experimental groups. All were housed for an appropriate interval with a small group of males of the stock (Wistar strain) in the colony in the Psychology Department at Washington University. The housing of the males with the females was done in such a way that the sires of the experimental offspring were also the sires of the control offspring.

Apparatus and Procedure

Deprivation. Following a positive test for pregnancy (by palpitation), the 40 experimental females were placed in a large decompression chamber and exposed to a simulated altitude of 30,000 ft. (equivalent percentage of oxygen, 6.21; sea level, 20.93) for 120 min. (The pressure in in. of mercury was 8.88 as compared to 29.92 in. at sea level.) The rate of ascent and descent was 3,000 ft. per min. The temperature was maintained at approximately 80° F. at all times during exposure.

Of the 40 animals submitted to the exposure, 12, or 30%, died in the chamber. Based upon observation through the window of chamber, it was thought that all of these animals died within the first 30 min. of exposure. One animal aborted and devoured her young during the first 10 min. of exposure.

Post-mortem examinations revealed that 2 of the 12 animals which died were not pregnant. The post-mortems revealed, further, that the ages of the fetuses at death ranged from an estimated 3 days to an estimated 8 days.

The living animals, following a short recovery period upon removal from the chamber, showed no overt physical disturbances. Moreover, this was true of the 2 deprived animals which died within 5 days and the 3 others which died with 10 days of exposure. Thus a total of 39% of the pregnant females died during deprivation or within 10 days following.

In the control group, 3 of the 40 animals died.

The total number of animals born to anoxic mothers was 180. Of these, 32 died or were destroyed shortly after birth by the mothers. The first 130 animals born were used in the behavior measures and the remaining discarded. To the control mothers, 172 young were born, and of these 35 died or were destroyed by the mothers. The first 130 born were used as the control group in the behavior measures.

The 130 young of the anoxic mothers were born on an average of 7 days following exposure, with the extremes being 2 and 11 days. Since the action of the oxygen deprivation upon the duration of gestation is not clear, no inferences can be drawn as to the exact fetal age at the time of exposure.

Behavior measures. The test situation utilized in measuring the animals' behavior included a straight water tank (a straight "swimway"), a fourteen unit T water maze, and a single unit T water maze.

The straight swimway served a dual purpose: to pretrain the animals for the mazes and to discern swimming speeds of both the anoxic and control groups. The tank used was 108 in. long, 8.5 in. wide, and 15 in. deep. The depth of the water in the tank was 9 in.

The animals were placed in a box suspended over the water at the starting end of the tank and then dropped 3.5 in. to the water through the hinged bottom of the box. The animals then swam to the ladder and goal platform (and warmth) at the opposite end of the tank. Each rat had 10 trials in this task on the 59th day after birth.

On their 60th day of age, the animals of both the control and the experimental groups were weighed. One hundred of each group were started on the multiple T maze.

The maze used was a 14 unit T maze (modified Stone pattern) filled with water to a depth of 9 in. In the scoring procedures, three types of errors were recognized and recorded, along with time and trials to criterion. The three types of errors scored were as follows: retracing in a section of the correct pathway, entering a wrong pathway, and entering either end of a cul-de-sac. Duplicated errors were individually recorded. Five gates were located at points as specified in an earlier report from this laboratory (Licklider & Bunch, 1946).

Each animal was given three trials a day until it reached a criterion of one errorless trial. Any animal spending more than 30 min. in the water in any one trial was removed from the situation and discarded from the experiment. On this basis, 3 anoxic animals and one control animal were discarded.

Thirty days after the original learning of the large maze had been completed, these animals relearned the maze to the same criterion (one errorless trial).

In the 30-day interval between the learning and relearning of the multiple unit maze, half (50) of the animals from each group of 100 learned a brightness discrimination problem in a single unit T water maze. The animals were run on the single

unit maze 11 days after they had reached the criterion on the 14 unit maze.

The length of the stem of the T maze was 42 in.; the length of each arm of the T was 18 in. The ends of the arms of the T were extended forward 6 in. parallel with the stem, making it impossible to see the escape ladder from the choice point. The water was maintained at a depth of 9 in. The temperature of the water was 5-6 degrees F. below the temperature of the room.

At the choice point of the T, a septum was placed separating the arms. A rod passed through this septum and fastened to the sides of the stem was used to hang the black and white gates. The positions of the gates were varied randomly. The escape ladder was placed on the side of the "correct" gate. The gates were free swinging, permitting the animal to return to the choice point after making a "bad" choice.

Each animal was given 80 trials at this task extending over an 8 day period, viz., 8 blocks of 10 trials.

An additional 30 anoxic and 30 control animals learned the discrimination problem without previous experience on the multiple unit T maze. These performances (scored by trials and errors, only) were used to differentiate the groups on the basis of transfer of training from the maze learning to the brightness learning.

Most animals completed the learning and the re-learning tasks by the 100th day of age. All animals were weighed at this time.

The schedule of testing with the various groups is shown in Table 14.

Results

The results are presented with reference to the following questions concerning the effects of prenatal exposure to oxygen deficiency upon learning and retention at maturity: (a) ability to learn and to retain the solution of a difficult maze problem, (b) relationship between the effects of prenatal anoxia on learning and the interval between the day of deprivation and the day of birth, (c) ability to learn a simple brightness discrimination problem, (d) transfer of training from one learning task to a second, and (e) inhibition (or facilitation) of interpolated learning upon relearning of the original task. Because of the imminent possibility of interaction effects, the analysis of the data also concerned questions of certain "side" effects of prenatal anoxia, that is, the effect on body weight, sex ratio, and speed of performance, specifically, speed in swimming the length of a straight tank.

TABLE 14

PROGRAM OF CONDITIONS OF LEARNING FOR EACH GROUP

Group	No.	Learning	Conditions of 30-Day Interval	Relearning
Control	100	Mult. T Maze	(50: rest) (50: learn B-W disc.)	Mult. T Maze
Anoxic	100	Mult. T Maze	(50: rest) (50: learn B-W disc.)	Mult. T Maze
Control	30	B-W discrimination		
Anoxic	30	B-W discrimination		

The effect of prenatal anoxia upon performance of learning and retention on difficult maze problem. The anoxic animals made 37% more errors in learning this maze to criterion (one errorless trial) than did the control group. The mean error score for the anoxic group was 226.33 and for the control, 164.61. The mean difference (61.72) was significant beyond the .01 level.

There were additional differences between the groups as related to the sex of the members. In superiority of performance (fewest errors), the control males were best; the control females, anoxic males, and the anoxic females followed in that order. The differences between the control males and the control females and between the control males and the anoxic males were significant beyond the .01 level. The difference between the control females and the anoxic females was significant beyond the .05 level

(one-tailed test; the order of difference was as expected).

The anoxic animals required 30% more trials to reach the criterion of mastery than did the controls. The mean trial score of the anoxic groups was 25.23, whereas the corresponding score for the control group was 19.35; the difference between these means was significant beyond the .01 level.

As with the error scores, differences in trial scores also existed between the groups as related to the sex of the members. The nature of the differences was about the same: the control males were superior (required the fewest number of trials); the control females, the anoxic males, and the anoxic females followed in that order. The differences between the two groups of males and between the two control groups (male and female) were statistically significant (.01 level).

TABLE 15

ANALYSIS OF ERROR DATA ON 14 UNIT MAZE

Group	No.	Group Mean	\bar{gMd}	Sex	t	p
Anoxic	100	226.33	15.37	Both	4.02	<.01
Control	100	164.61		Both		
Anoxic	39	241.85	24.86	F	1.89	<.05
Control	48	194.85		F		
Anoxic	61	216.41	18.82	M	4.24	<.01
Control	52	139.69		M		
Anoxic	61	216.41	25.17	M	1.01	>.05
Anoxic	39	241.85		F		
Control	52	136.69	18.40	M	3.15	<.01
Control	48	194.85		F		

TABLE 16
ANALYSIS OF TRIAL DATA ON 14 UNIT MAZE

Group	No.	Group Mean	σMd	Sex	t	p
Anoxic	100	25.23	1.82	Both	3.23	<.01
Control	100	19.35		Both		
Anoxic	39	27.05	2.81	F	1.59	>.05
Control	48	22.58		F		
Anoxic	61	24.07	2.31	M	3.33	<.01
Control	52	16.37		M		
Anoxic	61	24.07	2.98	M	0.90	>.05
Anoxic	39	27.05		F		
Control	52	16.37	2.30	M	2.70	<.01
Control	48	22.58		F		

The analysis of the time scores (time per trial) presents the same picture of inferiority of performance of the experimental animals. The anoxic group averaged 1.26 min. per trial as compared to the 1.16 min. averaged by the control group. The difference between these averages was statistically significant (beyond .01 level). Once again, further differences were noted when the two groups were subdivided according to the sex of the members. The control males were significantly faster than the control females (.05 level) and the anoxic males (.01 level).

As determined by performance on the multiple T maze, the differences between the groups were consistent for all measures used: for errors and trials to reach criterion and for average time per trial. In each instance, the control males were superior to the control females, anoxic males, and anoxic females. In each instance, differences between the control and experimental animals were attributable, largely, to dif-

ferences between the two populations of male animals.

Retention of the solution of this multiple unit maze after an interval of 30 days following original mastery differentiated, significantly, the control and the experimental groups.

For this comparison of behavior, only half (50) of each of the two groups were used. (The remaining 50 of each group were used for comparison of the effects of interpolated learning.) The design and results are shown in Tables 17 and 18.

In all cases the raw relearning scores of the control group were superior to the scores of the anoxic group. The latter made 21% more errors and took 14% more trials in relearning the maze than did the former, the control group. Only the differences in trial scores were significant statistically.

These results might be a function of the greater amount of practice for the anoxic group in the original learning problem. The

TABLE 17
COMPARATIVE RETENTION DATA FOR CONTROL AND ANOXIC ANIMALS UNDER
NORMAL ("REST") CONDITIONS FOR AN INTERVAL OF 30 DAYS
(Error Scores)

Group	No.	Conditions during Interval	Group Mean	σMd	t	p
Anoxic	50	Rest	30.26	6.06	1.11	>.05
Control	50	Rest	23.52			

TABLE 18

COMPARATIVE RETENTION DATA FOR CONTROL AND ANOXIC ANIMALS UNDER
NORMAL ("REST") CONDITIONS FOR AN INTERVAL OF 30 DAYS
(Trial Scores)

Group	No.	Conditions during Interval	Group Mean	σMd	t	p
Anoxic	50	Rest	6.50	0.88	1.77	<.05
Control	50	Rest	4.94			

anoxic animals made 46% more errors and required 34% more trials in the original learning situation. In any case, savings scores for the two groups were almost identical (median saving score in trials for control group, .80; median for anoxic group, .78).

Relationship between the effects of prenatal anoxia on learning and the interval between the day of deprivation and the day of birth. The data indicate significant differences in the ability to learn the maze as a function of the developmental stage at the time of oxygen deprivation. A summary of this analysis (analysis of variance) is given in Table 19.

A further review of the data revealed, however, that the bulk of the differences noted between the various experimental subgroups and between the total experimental group and the control group was contributed by a single experimental subgroup: the subgroup born 10 days after exposure (exposed, possibly, on the 13th fetal day). This subgroup differed significantly from each

of the other experimental subgroups and from the control group. No differences existed among the other experimental subgroups. In a further comparison between the control and the anoxic animals, with the subgroup born 10 days after exposure deleted, significant differences remained although the level of significance was reduced (from .01 to .05).¹⁰

Ability to learn a simple brightness discrimination problem. Two groups of 30 animals, experimental and control, were compared to determine the effect of prenatal anoxia upon simple black-white discrimination learning. Neither group had been used previously on any learning problem.

The control group made more correct responses in the block of 80 trials than did the experimental. The mean number for

¹⁰ Only the initial learning performance on the multiple maze could be evaluated in this fashion. Elsewhere the data were too meager to permit a subdivision according to fetal stage at time of deprivation.

TABLE 19

ANALYSIS OF VARIANCE OF ANOXIC GROUPS WITH RESPECT TO THE
INTERVAL BETWEEN DAY OF EXPOSURE AND DAY OF BIRTH

Age Groups	Measure	df	Sum of Squares	Variance	F	p
Between	Initial Learn.	8	280125.27	35015.66	2.69	<.01
Within	Errors	91	1183842.84	13009.62		
Between	Initial Learn.	8	3654.52	456.82	2.67	<.02
Within	Trials	91	15557.19	170.96		
Between	Relearning	8	2613.97	326.74	0.53	>.05
Within	Errors	91	62179.87			
Between	Relearning	8	165.84	20.73	1.27	>.05
Within	Trials	91	1506.91	16.38		

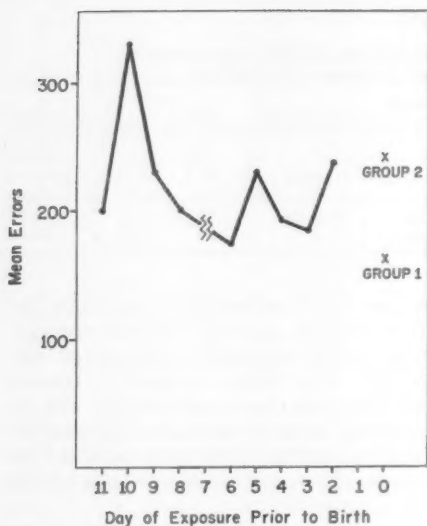


FIG. 11. Relationship between day of exposure to oxygen deficiency prior to birth and performance on the multiple T maze. For comparative purposes, the two points on the right, Group 1 and Group 2, are presented from the first study reported in this monograph.

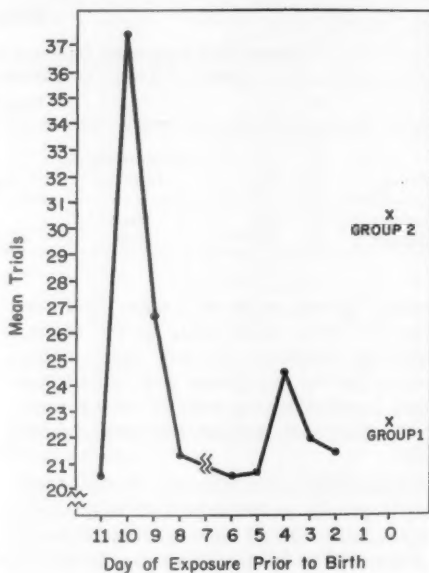


FIG. 12. Relationship between day of exposure to oxygen deficiency prior to birth and performance on multiple T maze. For comparative purposes, the two points on the right, Group 1 and Group 2, are presented from the first study reported in this monograph.

the control group was 50.93 and for the anoxic group, 47.17. These do not differ significantly, however ($p < .05$).

Transfer of training from one learning task to a second. Eleven days after they had reached the criterion on the multiple unit maze, 50 control and 50 anoxic animals were given 80 trials on the black-white discrimination task in the single unit water maze. The animals in both the anoxic and control groups, which were to be used on the discrimination problem, were selected

TABLE 20

ANALYSIS OF DATA ON SIMPLE DISCRIMINATION TASK: INITIAL LEARNING

Group	No.	Group Mean (CR)	σMd	t	p
Anoxic	30	47.17	2.05	1.83	$< .05$
Control	30	50.93			

prior to the beginning of the original learning on the complex maze. The selection was regarded as random with reference to the learning ability of the animals and to the subgroups related to the developmental stages at the time of deprivation.¹¹

The results of their performances on this second learning problem, measured in terms of correct responses in the block of 80 trials, are given in Table 21. The control animals were superior to the experimental in both situations, that is, in situations with and without the benefit of previous learning experience (see Figure 13).

On the black-white discrimination problems, after maze experience, the control and the anoxic groups made 51.30 and 45.18 correct responses, respectively. This differ-

¹¹ As it turned out, there were differences in the learning on the initial task and in the representation of the several experimental subgroups.

TABLE 21

ANALYSIS OF DATA ON SIMPLE DISCRIMINATION TASK: INTERPOLATED PROBLEM

Group	No.	Group Mean (CR)	Sex	σMd	t	p
Anoxic	50	45.18	Both	1.65	3.71	< .01
Control	50	51.30	Both			
Anoxic	22	43.56	F	2.08	3.21	< .01
Control	25	50.14	F			
Anoxic	28	46.80	M	2.50	2.56	< .01
Control	25	53.21	M			

ence in discrimination learning, as before, favors the control group. This difference was significant at the .01 level. It appears that the ability to utilize the experience on the complex maze in learning of a discrimination problem, as well as the ability to master the complex maze, initially, was inferior in the anoxic animals.

Effects of interpolated learning upon relearning the original task. The conditions

of interpolated learning were included in the experimental design in order to determine the relative susceptibility of the anoxic and the control animals to reproductive interference.

The brightness discrimination problem, the interpolated task in the 30-day interval between the learning and relearning of the multiple unit maze, did not inhibit the relearning performances; instead, both the anoxic and the control groups demonstrated facilitation on the second exposure to the maze. The differences in relearning scores between the anoxic "rest" group and the anoxic "interpolated" group was significant beyond the .05 level. However, a comparable difference (.05 level) existed between the two anoxic groups on the original learning of the multiple unit maze. This existed despite a random selection of animals prior to any learning experience whatsoever. This latter difference tends to disclaim the reality of any difference between the two groups as related to interpolated experience.

Facilitation instead of inhibition from the interpolated learning may be explicable on the basis of a number of factors: the relative dissimilarity between the maze and the discrimination tasks in the specific stimulus properties critical to learning and upon which interference might depend; the relatively high similarity between the two learning situations in terms of the general conditions of practice and performance involved, on which facilitation might depend; and the mere occurrence of daily exercise, handling, or performance, even apart from learning, in a similar motivating situation for almost

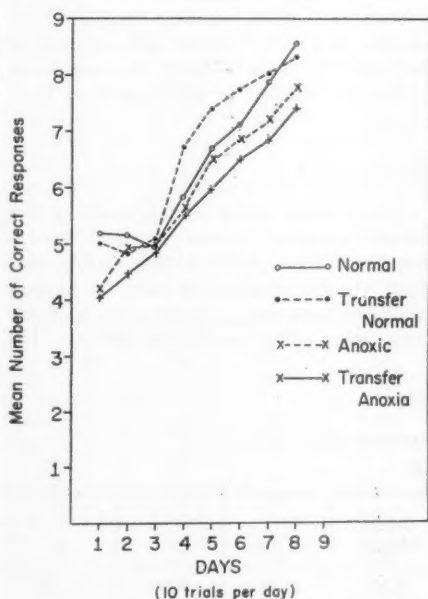


FIG. 13. Role of transfer of training among the control and experimental groups in the learning of the simple discrimination tasks.

TABLE 22
EFFECT OF INTERPOLATED LEARNING ON RELEARNING
(Errors)

Group	No.	Conditions during Interval	Group Means	σMd	t	p
Control	50	B-W discrimination	17.44	4.98	1.22	> .05
Control	50	Rest	23.52			
Anoxic	50	B-W discrimination	19.66	5.03	2.11	< .05
Anoxic	50	Rest	30.26			

$\frac{1}{3}$ of the 30-day interval which was not available to the "rest" groups.

"Side" Effects

Analysis of weight data. The mean weight of the anoxic group as a whole differs from the mean weight of the control group as a whole only on the basis of chance; no significant weight differences existed either at 60 or 100 days of age. A breakdown of the groups according to sex, however, revealed the significantly lighter weight of the anoxic females, as compared to the control females, at both dates. The anoxic and control males did not differ at any point. (See Table 24.)

Sex ratio. The subdivision of groups by sex was as follows:

	Multiple Y Maze		Brightness Discrimination		Total	
	Male	Female	Male	Female	Male	Female
Control	52	48	15	15	67	63
Anoxic	61	39	20	10	81	49

Although in most instances there were more males than females, with the superiority in

frequency magnified for the experimental groups, no significant differences existed.

Speed of swimming in the straight tank.

From the absence of any differences in swimming speed, one may conclude that differences in learning performances of the anoxic and control groups cannot be attributed to this factor. The mean of the control group was 6.43 sec. and, of the anoxic group, 6.74 sec. per trial for the block of 10 trials. (The critical ratio on these data of .92 was insignificant.)

Even though the anoxic females were smaller than their control counterparts, as indicated by body weight, they were not materially affected in this aspect of locomotion.

Discussion

The primary result of this study is that animals exposed to severe oxygen deprivation for a short period of time during their fetal life are impaired at maturity in their ability to learn and to retain (after a 30-day interval) a complicated maze pattern. The

TABLE 23
EFFECT OF INTERPOLATED LEARNING ON RELEARNING
(Trials)

Group	No.	Conditions during Interval	Group Means	σMd	t	p
Control	50	B-W discrimination	4.22	0.73	0.99	< .05
Control	50	Rest	4.94			
Anoxic	50	B-W discrimination	4.00	0.79	3.16	> .01
Anoxic	50	Rest	6.50			

anoxic animals were inferior to the control animals in all measures of ability to learn and to retain the maze and to learn a discrimination problem, although in some instances this inferiority was not a statistically significant one.

The results of the initial learning on the 14 unit water maze were in the same direction as those results reported by Hurder and Sanders (1953) and Meier and Bunch (1950). In each of those studies, however, the deprivation occurred shortly after birth. In general, the results of this experiment indicate greater differences between the anoxic and control groups than in the other studies mentioned. It is impossible at this time to state the basis of the differences in magnitude between this and the other studies; it could be the prenatal (especially deprivation at a particular developmental stage) versus neonatal conditions; it could be a difference in the severity of the deprivation, *per se*; and so on.

Meier and Bunch reported that anoxic animals do not perform as well, according to some measures, as control animals on a single T water maze with respect to transfer of training in learning a position habit. It can be added from this study that animals deprived of oxygen prenatally: (a) do not perform as well as control animals on a single T water maze when ability to discriminate brightness is being measured and

(b) show less ability to profit from previous learning of a maze problem.

The results on the relearning of the maze were not consistent with those reported by Meier and Bunch (1950), Windle and Becker (1943), and others reported in this monograph. Meier and Bunch reported that learning was relatively unaffected by neonatal deprivation, but that retention following 2 or 14 days was markedly altered. This study found that original learning is much more severely impaired than is retention. Retention here was measured by relearning (Meier & Bunch used both relearning and reversal of habit), which was started 30 days after the completion of the original learning. It is possible that the presence of significant differences may be a function of the length of the interval over which retention was measured.

A factor of unknown importance was the difference between the groups when subdivided by sex. Although not significant, a difference did exist in the frequency distribution of females and males between the control and experimental groups. In every instance where a comparison was possible, the males considerably outnumbered the females in the anoxic groups, but were almost equal in number in the control groups. Along with this, in each instance where significant differences were found between the experimental and control groups, the

TABLE 24
ANALYSIS OF WEIGHT DATA

Group	No.	Age (days)	Sex	Group Means (grams)	σMd	t	p
Anoxic	74	60	Both	167.58	5.36	1.25	> .05
Control	83	60	Both	174.27			
Anoxic	36	60	F	137.94	3.45	4.03	< .01
Control	42	60	F	151.83			
Anoxic	38	60	M	195.66	5.70	0.28	> .05
Control	41	60	M	197.24			
Anoxic	100	100	Both	245.99	6.78	0.02	> .05
Control	100	100	Both	246.11			
Anoxic	39	100	F	195.28	4.98	2.94	< .01
Control	48	100	F	209.93			
Anoxic	61	100	M	278.60	6.41	0.10	> .05
Control	52	100	M	279.21			

differences were largely explicable by the differences between the two populations (control and experimental) of males. This, however, was in spite of the consistently inferior performances of the anoxic females as compared to the other groups.

Although the anoxic females were significantly lighter than the control females (the only significant weight difference), the measures which would be expected to show consequential differences in behavior failed to do so. No differences were found in the swimming speeds in the straight tank. Differences in physical dimensions seemingly played an insignificant part in the explanation of differences in behavior.

In the light of these facts, there is a possibility that the learning data presented in this study were more favorable to the anoxic group than the differences discussed above would indicate. Possibly, one group, the females, was selected *against*. This group, as suggested by the nature of the behaviors found here, would have been even more drastically affected had a comparable ratio of females to males been attained for the anoxic group as was attained for the control group. Such an equitable distribution by sex, in all likelihood, would have made differences between the anoxic and control groups even greater.

The differences between the several experimental subgroups (subdivided by fetal age at the time of deprivation) are difficult to evaluate at this time. If one could make the necessary adjustments for species, developmental rates, severity of deprivation, and the time and nature of measurement, these differences might be found to be of the same basis as those described by Meier and Menzel (1955) in chicks. In their study, oxygen deprivation (LD_{50}) at the end of 8 days of incubation (21 days is "full term") was particularly effective in producing behavioral dysfunctions. The concepts offered by them in explanation of the results dealt with "critical period." In this usage, the concepts had been generalized from the critical periods in the growth of tissues and organs to critical periods in the growth of mature behavior patterns.

Several incidental, unquantified observations should also be added to the description of the experimental animals in this study. For the most part, none of the gross physical abnormalities reported by Ingalls (1947a, 1947b) in his experiments with mice were found with the rats in this study. It was noted, however, that 5 anoxic animals (of the 180 born) were blind and, consequently, were not used in the behavior measures.

Certain behavioral "peculiarities" were observed in the experimental animals. They tended to be more "skittish" and fractious than the controls. In their mastery of the several learning measures used, they tended to be more variable (this was reflected in the large group variances). These characteristics were similar to those observed in the study on neonatal anoxia in cats, reported by Meier (1953). Plausibly, these behaviors can explain differences in learning ability, especially since some of the differences tended to disappear as the animal had more experience in learning situations. That is, a deficit in learning ability may not be operative in these studies so much as a factor of heightened emotionality.

Summary and Conclusions

This experiment was conducted to measure the effects of prenatal anoxia on maze learning ability in rats. Forty pregnant rats were exposed in a decompression chamber at an altitude of 30,000 ft. (6.21% equivalent oxygen) for a period of 2 hrs. The rate of ascent and descent was 3,000 ft. per min. A total of 130 offspring of the exposed females were used in this study as experimental Ss, and their performance on two different maze problems was compared to the performance of 130 offspring of 40 control animals.

The maze problems consisted of a 14 unit modified Stone T water maze on which control and experimental animals were required to reach a criterion of one errorless trial, and a single unit T water maze on which number of correct responses in 80 learning trials was the measure obtained.

Data were also procured for: retention as measured by relearning after a 30-day interval; transfer of training from the 14 unit T maze to the black-white discrimination problem on the single T maze; and the effects of learning an interpolated problem (the black-white discrimination problem) on relearning the original problem.

In addition to the above data, other variables considered were: weight of control and experimental animals at 60 and 100 days of age, speed of swimming in the two groups as measured by a straight water tank, the number of days before birth the anoxic animals were exposed to oxygen deficiency, and possible physiological disturbances caused by anoxia.

The following are conclusions based on the data obtained:

1. Prenatal anoxia severely affects an animal's ability to learn a 14 unit T maze. The animals deprived of oxygen for a short period during fetal life perform at maturity in an inferior manner to a control group in terms of errors, trials, and time. The differences were found to be significant beyond the .01 level.

2. Retention as measured by trials to relearn the 14 unit water T maze was significantly different between the control and experimental groups.

3. Anoxic animals which made the poorest learning records were those whose period of oxygen deprivation preceded birth by approximately 10 days.

4. The learning on a simple black-white discrimination problem on a single T water maze significantly differentiated the learning abilities of 30 anoxic and 30 control animals.

5. Positive transfer from the original learning problem to the simple black-white discrimination problem was slightly but significantly greater in the controls than in the anoxic animals.

6. The black-white discrimination problem, when interpolated between original learning and relearning of the 14 unit T maze, led to reproductive facilitation in both groups. The anoxic animals appeared to

profit more from the interpolated learning than did the control group.

7. Generally, observable physical deformities were at a minimum. The only recognized deformity was blindness in five anoxic animals.

8. Female anoxic animals weighed significantly less than did female control animals; anoxic males did not differ from control males, even though the anoxic males were primarily responsible for significant differences between control and experimental groups on measures of behavior.

9. Speed of swimming in a straight tank was not seriously affected by prenatal oxygen deprivation.

10. The data do indicate that impairment in learning ability is a function of prenatal exposure to oxygen deficiency.

Histological Analysis

Following the completion of the maze tests, a limited sample of the animals was sacrificed and the brains prepared for histological analysis. The selection of the animals was made on the basis of their performances on the multiple unit maze. This sample included the two best and the four poorest performers of the control group and the four best and the six poorest performers of the experimental group (without regard to fetal age at the time of oxygen deprivation). In all, 16 Ss were evaluated in this fashion.

In the data obtained, no significant histological trend, as related to prenatal anoxia, was apparent. No qualitative differences (size and shape of cells, etc.) could be seen between the control and experimental animals. While there were considerable variations in thickness of the cortex of the cerebrum and in the relative cell counts in the same structure, these seemed to be due, largely, to variations within the individual animal and in no way correlated with performance on the multiple unit maze.

Should prenatal anoxia cause cell anomalies or death, these techniques on this selected sample did not show them—certainly, these techniques did not show them

to be functionally related to the measured behavior of the Ss.

(For a more extensive discussion of the techniques used, their rationale, and the results gained, see Appendix B.)

Part II

Two questions raised by the first half of this study made a replication of the experiment, in part, mandatory. (a) Since the dates of conception were not known, the fetal ages at the time of oxygen deprivation could only be roughly estimated. Moreover, these estimates, based on the number of days prior to birth the fetuses were deprived, seemed risky, certainly questionable. Can this form of insult, an exposure to decompression with the attendant lack of oxygen, accelerate or, even, decelerate, prenatal development and alter, thereby, the time of parturition? (b) The maximal effect of the deprivation was upon those animals born 10 days after the insult. The effect upon the young born 9 days or less or 11 days or more after the insult was markedly less obvious. Could this in reality be a "critical period" at which time the developing organism is uniquely sensitive to changes in available oxygen? Could there be other critical periods at earlier fetal ages? (Apparently fetal ages less than 11-12 days were not sampled in the first part of this study.) These matters formed the basis of a replication in which the fetal ages tested would represent the entire period of gestation.

Subjects

According to plan, 66 pregnant female rats were to be used in this part of the study. Each of the days of gestation, from 1-22, were to be represented by 3 of the pregnant rats. Unfortunately, this plan met with some difficulty; only 62 pregnant Wistar rats were received from a local dealer at the appointed time. This meant that several of the fetal age groups were not fully represented (see Table 26). (The appearance of a vaginal plug was accepted by the breeder as positive indication of mating behavior.) Further, 2 of the females of the group which had been bred 22 days prior to their receipt in the laboratory dropped their young within minutes of the scheduled time of the oxygen deprivation. Consequently, only 21, rather than 22, intervals in the fetal development were examined in this study.

Apparatus and Procedure

Deprivation. Forty-one of the pregnant females, 2 from each of the 21 groups of 3, were placed in a large decompression chamber. (There were exceptions; these are indicated in Table 25. Where both the control and experimental groups could not be included in a fetal age group, because of small size, only the experimental group was represented. As a result of this, there were 41 experimental pregnant animals and 19 control.) The pressure within the chamber was reduced until a simulated altitude of 30,000 ft. (8.88 in. Hg.; 6.21% equivalent oxygen) was reached. This simulated altitude was reached in 15 min. The animals were maintained under these conditions for 120 min., after which time the pressure was returned to normal. This, too, required 15 min. All in all they were under some degree of decompression and oxygen deprivation for 150 min.

The females were placed in individual cages and the respective dates of delivery of their litters were recorded.

Of the 254 young born to the experimental females and the 124 born to the controls, 108 were selected for testing purposes. Since it was recognized that litter differences might be a major confounding factor in this study (with the small number of litters at each fetal age), the experimental offspring were divided into five groups. These groups, with their numerical size, were:

Group I: Deprived 2-5 days prior to birth: 14 Ss.

Group II: Deprived 6-9 days prior to birth: 14 Ss.

Group III: Deprived 10-13 days prior to birth: 19 Ss.

Group IV: Deprived 14-17 days prior to birth: 20 Ss.

Group V: Deprived 17-21 days prior to birth: 16 Ss.

Control Group: 25 Ss.

(Originally, it was planned that there be but 14 Ss in each of the 5 experimental groups and 25 in the control group. As the running of the Ss on the maze was more rapid than anticipated, the size of the last three experimental groups was increased.) The Ss for these groups were selected with the view of representing as many individual litters as possible. No consideration was given to the sex ratios of the several groups. In all, there were 83 Ss from the asphyxiated mothers and 25 from the control used for testing purposes.

Behavior measures. The 108 animals were permitted to mature under normal laboratory conditions until the 64th-67th days at which time they were pretrained on the straight swimway used in the first half of this study. After 10 trials on the swimway (completed within a single session) the animals were run on the multiple unit water maze (also used in the first portion of the study) until they reached the criterion of one errorless per-

form
3 tr
and
Res
mal
3 d
pos
exp
in t
enti
25)
peri
C
exp
Aga
fail
exp
does

Totals

* Died

formance. On this apparatus the Ss were given 3 trials each day. Besides trials to criterion, time and error scores were recorded.

Results

Vital statistics. Of the 41 pregnant animals exposed to the deprivation conditions, 3 died during or immediately following exposure. Two more died within 5 days of the exposure. There seemed to be no pattern in these deaths; deaths occurred within the entire range of gestation ages (see Table 25). (No control females died within this period.)

Only 44 litters were delivered; 30 to the experimental group and 14 to the control. Again, no pattern can be found in these failures; in fact, the notion that some of the experimental animals could have aborted does not seem to be tenable, since the num-

ber of failures for the two groups are almost the same (5, control; 6, experimental).

Although there was a difference in the average length of the gestation periods in favor of the control group (0.42 days), this was not found to be significant. (See Table 26.) More than likely, this difference is considerably less than the errors inherent in the setting of the date of mating and the date of delivery. A cursory inspection of Table 28 will reveal that only one delivery seemed to have an unusually short gestation period and only two longer (considerably) than usual. Other than these, the control and the experimental groups were very much alike.

Again, although there were differences in litter size at birth and at 65 days of age in favor of the control group, statistical significance of these differences was lacking.

TABLE 25

VITAL STATISTICS: I

(Breeding and delivery data on the control (C) and experimental (X) females)

Mating Date	No. in Group	No. of Litters		No. of Deaths		Failures to Drop	
		C	X	C	X	C	X
8-1	1	1					
8-2	3	1	2				
8-3	3	1	2				
8-4	3	1	1		1		
8-5	1		1				
8-6	2		1		1		
8-7	3		2			1	
8-8	3	1	2				
8-9	3	1	2				
8-10	3	1	2				
8-11	3		1				
8-12	3	1			1*	1	1
8-13	2		2				1
8-14	3	1	1		1*		
8-15	3	1	2				
8-16	3	1	2				
8-17	3	1	2				
8-18	3	1	2				
8-19	3		2			1	
8-20	3				1*	1	1
8-21	3		1			1	1
8-22	3	1					2
Totals	60	14	30		5	5	6

* Died during the oxygen deprivation. The others died within 5 days of exposure.

TABLE 26

VITAL STATISTICS: II

Litter and Viability Data with Respect to Dates of Mating and Parturition

Birth Date	Breeding Date	Length of Gestation (days)	Group	No. Born	No. Dead at Birth	No. Dead at 65 Days	No. Alive at 65 Days	Dispensation
8-23	8-1	22	C	14			14	6 to C
8-25	8-2	23	X	9			9	5 to I
	8-2	23	C	9			7	
	8-3	22	X	5	1	1	3	1 to I
8-26	8-2	24	X	8	4	3	1	
	8-3	23	X	3		3		
	8-3	23	C	9		1	8	2 to C
8-28	8-4	24	X	7	3		4	2 to I
	8-4	24	C	12			12	4 to C
	8-5	23	X	6			6	3 to I
	8-6	22	X	6			6	3 to I
8-30	8-7	23	X	13	1	2	10	4 to II
	8-7	23	X	11		10	1	
	8-8	22	C	3		1	2	1 to C
	8-8	22	X	6		6		
	8-8	22	X	11	1	1	10	4 to II
8-31	8-9	22	X	10			10	6 to II
	8-9	22	X	11		8	3	6 to 2
9-1	8-9	23	C	9	1	1	7	2 to C
9-2	8-10	23	C	3	2	1		
9-3	8-10	24	X	12	1	2	9	6 to III
	8-11	23	X	10		7	3	
9-4	8-12	23	C	10		5	5	2 to C
	8-15	20	X	14	2	1	11	5 to III
	8-13	22	X	11		2	9	6 to III
9-5	8-13	23	X	7			7	2 to III
	8-14	22	C	10			10	3 to C
9-6	8-10	27	X	7	3		4	3 to IV
	8-14	23	X	11	5	3	3	3 to IV
	8-15	22	C	6		6		
9-7	8-15	23	X	11		2	9	5 to IV
9-8	8-17	22	C	8		6	2	
9-9	8-16	24	X	3		1	2	1 to IV
	8-16	24	X	13		1	12	8 to IV
	8-17	23	X	8	1	7		
	8-18	22	C	10	2	8		
9-10	8-16	25	C	9			9	3 to C
	8-17	24	X	3	3			
	8-18	23	X	9	1	1	7	2 to V
9-11	8-19	23	X	5			5	5 to V
9-12	8-19	24	X	4		4		
9-13	8-18	26	X	13	1		12	5 to V
	8-21	23	X	7	2	1	4	4 to V
	8-22	22	C	9		2	7	2 to C
Number (control)				121	6	32	83	
Number (experimental)				254	28	66	160	
Mean (control)				8.64			7.54	
Mean (experimental)				8.47			6.40	

Any differences between the two groups in the matter of litter size was attributable to difference in the litters at the time of birth. Further, there was no trend in the size of the litter and the gestation age at the time of deprivation.

In summary, with the procedures used in which the breeding and deliveries were checked regularly once every 24 hrs., no differences could be detected between the control and the experimental groups of animals. Possibly, more frequent inspections could have shown differences between the two groups.

Behavioral measures: Pretraining and maze performance. The performances of the animals were analyzed first by treatment, that is, by age at the time of deprivation, then by sex. As on most of the criterion measures the data were nonnormal; these analyses were completed with the use of the Kruskal-Wallis (Siegel, 1956, pp. 184-193) one-way analysis of variance. This particular analysis utilizes the data by ranks rather than by the actual raw data. (In these instances there was little loss of statistical power as compared to the usual F tests. Were the assumptions of F met, the asymptotic efficiency of the Kruskal-Wallis would be 95.5.)

The median time scores for the 10 trials on the straight swimway were not statistically significant whether analysed by age at deprivation or by sex. The median scores were:

	C	I	II	III	IV	V	Totals
Males	48.4	51.4	63.9	50.0	51.3	63.5	54.3
Females	52.4	53.8	72.0	51.3	53.4	49.8	54.7
Totals	50.3	52.7	67.4	50.6	52.6	58.3	

The variance H for treatments was 3.47; for sex, 0.00. (For significance at the .05 level, H would have to equal or exceed 12.83 for treatments and 5.02, for sex.)

On the maze itself, seven different criterion measures were evaluated. These were: (a) trials to criterion, (b) errors to criterion, (c) errors per trial, (d) errors on the first three trials, (e) time to criterion, (f) time per trial, and (g) time on the first three trials.

Trials to criterion: no differences were found between groups on the number of trials required to reach the criterion of mastery (one errorless performance). The median performances were:

	C	I	II	III	IV	V	Totals
Males	55.9	44.6	49.6	47.3	48.7	60.2	51.8
Females	51.4	67.1	69.0	49.9	48.0	73.3	57.5
Totals	53.7	57.4	58.0	48.5	48.3	65.1	

Both the H for treatment, 3.60, and the H for sex, 0.92, were clearly below the required values (12.83 and 5.02, respectively) for significance at the .05 level.

Errors to criterion: although the various treatment groups could not be differentiated on this measure of performance, the males and females were found to be significantly different ($p < .05$) on the number of errors made in reaching mastery. The males were vastly superior to the females in all treatment groups.

	C	I	II	III	IV	V	Totals
Males	58.2	47.8	57.5	47.1	30.9	42.2	47.5
Females	59.8	62.1	86.2	59.2	50.4	68.7	62.4
Totals	59.0	56.0	69.8	52.8	40.6	52.1	

H for treatment was 7.95; for sex, 6.09. (For significance at the .05 level this latter value must equal or exceed 5.02.)

Errors per trial: again, no differences were found between groups due to treatment on the basis of the ratio of total errors to total trials to reach mastery for each S . Nor were differences found in performance due to sex.

	C	I	II	III	IV	V	Totals
Males	56.2	63.4	67.9	56.8	31.7	36.4	50.9
Females	63.9	48.6	65.1	67.7	61.8	35.2	58.5
Totals	59.9	54.9	66.7	62.0	46.7	35.9	

H for treatments was 10.8; for sex, 1.57.

Errors on the first three trials: since one of the S s mastered the maze in three trials, the data for all were analyzed through this point. By this we hoped to get a common base line for all S s unbiased by "obvious relationships" as would be expected to occur in the derived measures of behavior, e.g., errors per trial, time per trial.

The groups did differ significantly ($p < .05$) by treatments on the number of errors made in the first three trials. No differences were shown in behavior attributable to sex.

	C	I	II	III	IV	V	Totals
Males	51.8	71.3	69.3	47.0	37.5	42.9	51.4
Females	48.6	67.0	82.2	63.9	49.9	47.2	58.0
Totals	49.8	68.8	74.8	55.0	43.7	44.5	

H for treatments was 13.41; for sex, 1.19. (For significance at the .05 level, H must equal or exceed 12.83.)

Time to criterion: again, the groups differed significantly ($p < .05$) by treatments, now in the total time required for the mastery of the task. Again, no differences in performance were found attributable to sex differences.

	C	I	II	III	IV	V	Totals
Males	65.5	57.8	53.1	34.3	37.3	50.6	49.9
Females	56.3	74.5	81.2	50.9	39.3	72.0	59.6
Totals	61.1	67.3	65.1	42.2	38.3	58.6	

H for treatments was 13.65; for sex, 2.60.

Time per trial: as might be expected from the significant differences in total time and the lack of significance in total trials (although the two trends were the same), a derived measure based upon these two also pointed to significant differences ($p < .01$) between the groups by treatment. No differences in performance were related to differences in sex.

	C	I	II	III	IV	V	Totals
Males	67.4	71.8	62.8	58.7	43.1	45.2	49.5
Females	64.3	74.5	77.7	35.7	29.2	36.6	60.0
Totals	65.9	73.4	69.1	46.6	36.1	39.8	

H for treatments was 23.06; for sex, 3.03. (For significance at the .01 level, H for treatments must equal or exceed 16.75.)

Time on the first three trials: the groups, as classified by dates of delivery after the period of oxygen deprivation, differed significantly ($p < .02$) on the times to complete the first three trials. Once more, no differences in time scores were attributable to differences in sex.

	C	I	II	III	IV	V	Totals
Males	65.7	72.1	67.4	35.6	46.0	49.0	54.9
Females	51.4	78.3	68.6	49.4	42.1	39.4	54.0
Totals	58.8	75.6	67.9	42.1	44.0	45.4	

H for treatments was 16.00; for sex, 0.02. (For significance at the .02 level, H for treatments must equal or exceed 15.09.)

Discussion

On the face of things, the data on duration of gestation justifies an extrapolation of the gestation age at the time of deprivation from the number of days which this deprivation preceded birth, as might have been done in the first part of the study. However, there are several qualifications that must be considered before concluding that this bout of oxygen deprivation has no effect on the duration of gestation. The first deals with the procedures used in this study for the determination of the data of breeding and delivery. Possibly, a closer check could have been made on the time of mating. From the breeder, we learn that his procedures entailed a maximum error of 12 hrs. Certainly, a more frequent check could have been made in the designation of the date of delivery. Routine 12 hr., rather than 24 hr., observations would have been feasible. In all, more frequent observations could have materially reduced the procedural variance which in this instance was so much larger than the group differences.

Second, work in the Vanderbilt Laboratory has shown a regular variation in the date (and hour) of hatching of chicks which had been exposed to an acute oxygen deficiency during incubation. We have found that a median lethal deficiency at the end of the 8th day of incubation (full incubation: 21 days) will delay hatching by an average of 12 hrs., whereas deprivation at the end of the 19th day will accelerate hatching by 6 hrs. (Again, chicks are uniquely suited to this type of research; here, because the determination of date of "birth" is both easier and more meaningful for the individual *S*.)

Although significant differences were detected among treatment groups only on the last four measures, their pattern of performance was consistent on all seven. The poorest performances were observed in animals born 6-9 days following oxygen deprivation (gestation age: 13-16 days). The

best performances were observed in those animals born 14-17 days following deprivation (gestation age: 5-8 days). In broad outline, these data in part 2 are consistent with those of part 1.

In some respects, however, the notion of critical periods was not materially strengthened. A cursory observation of the group trends, both on separate and pooled measures, reveals an increased susceptibility to impairment by this insult as the animals mature to parturition. Nevertheless, it should be pointed out that the performances of the animals deprived very *early* in gestation were as different from the controls as those of the animals deprived very *late*. On most measures, the control group stood in between these two age groups.

Conceivably, some factor could be affected in early gestation which would tend to "improve" the Ss' behaviors, as measured here. (At least, the time scores were shortened, the error scores were kept to a minimum, etc.) Likewise, some factor could be affected in middle and late gestation which would tend to "downgrade" the Ss' behaviors, again, at least as measured here. Given the two separate functions for these factors, one could justifiably speak of two critical periods centering about the two phases of the animals' prenatal development.

Summary and Conclusions

This replication was made to check on the effect of oxygen deprivation on the length of gestation and the possibility of "critical periods" earlier in gestation than noted in the earlier part of this study.

Forty-one pregnant Wistar rats were exposed to a simulated altitude of 30,000 ft. for 120 min. These animals represented, in groups of two (with irregularities, sometimes less), 21 stages in the fetal development of the rat. Following exposure, the animals were placed in individual cages and the dates of delivery recorded.

The progeny of 19 pregnant animals of comparable gestational development were the controls.

From the data collected, no differences

would be ascribed to the experimental and control groups with reference to duration of gestation, size of litter at birth, and size of litter at time of running on the behavior measures.

At the age of 64-67 days the animals were run 10 trials on a straight swimway following which they were run on a 14 unit water maze to the criterion of one errorless trial. On the basis of the performances on these apparatus we can conclude:

1. There were no differences in median time scores for the 10 trials of the straight swimway. This might be construed to mean that there were no differences in activity levels between the experimental and control groups.

2. No differences were noted between the groups on the number of trials or errors to reach the criterion of one errorless performance. Further, no differences were found in the number of errors per trial made by the two groups of Ss.

3. Significant differences were found in the time measures of behavior, the time to criterion ($p < .05$), and the time per trial ($p < .01$).

4. As one S (control) mastered the maze in three trials, analyses of the behaviors of all of the Ss were made through this point. There were significant differences on both the number of errors made in these three trials ($p < .05$) and in the time required for completion of these trials ($p < .02$).

5. Consistently, Group II made the poorest performances. This group was comprised of animals born 6-9 days following the oxygen deprivation (gestation age: 13-16 days). This tends to confirm the findings of part 1 of this study.

6. Consistently, Group IV made the best performances. This group was comprised of animals born 14-17 days following the deprivation. (Gestation age: 5-8 days.) Conceivably, the performances of this group and of Group II indicate the existence of two unique "critical periods." As measured in this study, an insult in one period may have a facilitative effect whereas an insult in the other may have a deleterious effect on maze learning.

SUMMARY AND CONCLUSIONS

The three experiments reported in the present study are primarily concerned with the effects of natal and prenatal anoxia upon subsequent behavior, and upon learning and retention at maturity in rats and in cats.

Results are reported on the effects of a single exposure to anoxia, at birth in experiment 1 and at different stages during the prenatal period in experiment 3, on the ability of white rats at maturity (a) to learn a difficult and reliable maze problem (14 unit multiple T pattern), (b) to utilize the previous learning of the maze problem in the later learning of a simple discrimination task (transfer of training), (c) to retain the maze habit over an interval of 30 days under normal conditions during the interval, (d) to retain the maze habit over an interval of 30 days under conditions designed to produce retroactive inhibition, (e) to extinguish a bar pressing response recently acquired, and (f) to reverse a simple position habit shortly after its mastery. In all, data are reported on approximately 474 white rats in the learning and retention studies. Approximately one half were control and one half were anoxic animals.

Behavior data are presented in experiment 2 on cats showing the effects of a single exposure to anoxia at birth upon their ability to learn, as measured in the following battery of tasks: Guthrie puzzle box, single unit T maze, double alternation problem, and a brightness discrimination problem. Also investigated, but without positive results, were the effects of natal anoxia upon adult EEG patterns in the cat and the influence of prenatal anoxia upon gestation period and upon structural brain damage in adulthood in the rat.

The experiments have shown the following results:

1. Natal Anoxia in Rats

a. Thirty minutes of oxygen deprivation (2.91% equivalent oxygen) following birth had no significant effect upon learning or retention in rats of a difficult maze problem

nor upon the extinction of a bar pressing habit.

b. Sixty minutes of oxygen deprivation under similar conditions, on the other hand, produced significantly inferior learning and retention of the multiple unit maze, in terms both of number of errors made and number of trials required to reach the criterion of mastery.

c. Sixty minutes of the severe oxygen deprivation did not significantly affect the learning of a simple position response but it did have a deleterious effect upon the reversal of that habit.

2. Natal Anoxia in Cats

a. Rapidity of response in the puzzle box was significantly decreased.

b. Poorly adapted responses, such as "stereotypy," were significantly increased in certain discrimination tasks: learning a position response rather than the required discrimination.

c. A reduced ability to sustain a symbolic solution once the solution had been reached.

d. There was no indication that oxygen deprivation affected the ability to learn a brightness discrimination where there was close spatial relationship and temporal proximity of response between instrumental objects and reward.

3. Prenatal Anoxia in Rats

In Part I, in which 40 pregnant rats were exposed to 6.21% equivalent oxygen for two hrs. and 130 offspring were compared with 130 offspring of 40 control mothers, the effects of prenatal anoxia upon learning, retention, and transfer were as follows:

a. The animals deprived of oxygen during fetal life performed in a significantly inferior manner to control animals in terms of errors, trials, and time required to learn the multiple unit maze.

b. Retention of the maze problem, as measured by trials to relearn, was significantly impaired.

c. Learning the simple black-white discrimination problem was significantly poorer in the anoxic as compared with the control animals.

d. Slight but significantly less positive transfer was shown by the anoxic than by the control animals from the maze problem to the black-white discrimination problem.

e. Anoxic animals which made the poorest learning records were those whose period of oxygen deprivation preceded birth by approximately 10 days.

f. Retroactive inhibition in the retention of the maze problem was not produced by the interpolated discrimination learning, in either the anoxic or control animals.

g. Observable physical deformities were at a minimum; the female anoxic animals weighed significantly less than did female control animals.

Part II replicated Part I with the modification that the single exposure of pregnant females of 6.21% equivalent oxygen for a period of two hrs. was studied at 21 different stages in the gestation period. The comparison of the prenatal anoxic group with control animals showed the following results:

1. There were significant differences between anoxic and control animals indicating

superiority of the latter in the following measures in learning the maze problem: the time to criterion, the time per trial, and number of errors made on the first three trials. There were no effects of anoxia in the number of trials or errors made in learning the problem to mastery.

2. The gestation age at which oxygen deprivation occurred was a significant factor with respect to maze learning ability. Animals born 6-9 days following deprivation (gestation age: 13-16 days) consistently made the poorest performance. Animals born 14-17 days following deprivation (gestation age: 5-8 days) consistently made the best performance.

3. Vital Statistics: of the 41 pregnant animals exposed to the deprivation conditions, three died during or immediately following exposure and two more died within five days of the exposure. Mortality did not seem to be related to a particular gestation age at time of deprivation. No control females died during this period.

4. No differences were found between anoxic and control groups regarding duration of gestation period.

REFERENCES

- APGAR, VIRGINIA, GIRDANY, B. R., MCINTOSH, R., & TAYLOR, H. C., JR. Neonatal anoxia: I. A study of the relation of oxygenation at birth to intellectual development. *Pediatrics*, 1955, **15**, 653-662.
- ARMITAGE, S. G. The effects of barbiturates on the behavior of rat offspring as measured in learning and reasoning situations. *J. comp. physiol. Psychol.*, 1952, **45**, 146-152.
- BECKER, F. R., & DONNELL, W. Learning behavior in guinea pigs subjected to asphyxia at birth. *J. comp. physiol. Psychol.*, 1952, **45**, 153-162.
- BRESLIN, F. D. The effect of oxygen lack upon physical and neurological development in the chick. *Amer. J. Ment. Defic.*, 1956, **60**, 595-602.
- CHARLES, MARGARET S., & FULLER, J. L. Developmental study of the electroencephalogram of the dog. *Electroenceph. clin. Neurophysiol.*, 1956, **8**, 645-652.
- DESPERT, J. L. Anxiety, phobia, and fears in young children. *Nerv. Child*, 1946, **5**, 5-23.
- EDWARDS, A. L. *Experimental design in psychological research*. New York: Kinehart, 1950.
- FAZEKAS, J. F., ALEXANDER, F. A. D., & HIMWICH, H. E. Tolerance of the newborn to anoxia. *Amer. J. Physiol.*, 1941, **134**, 281-287.
- FISHER, R. A. *Statistical methods for research workers*. London: Oliver & Boyd, 1946.
- GIBBS, F. A., & GIBBS, ERNA L. *Atlas of electroencephalography*. Vol. I. *Methodology and controls*. (2nd Ed.) Cambridge, Mass.: Addison-Wesley, 1950.
- GLASS, H. G., SNYDER, F. F., & WEBSTER, E. The rate of decline in resistance to anoxia in rabbits, dogs, and guinea pigs from the onset of viability to adult life. *Amer. J. Physiol.*, 1944, **140**, 609-615.
- GOMEZ, L., & PIKE, F. H. The histological changes in nerve cells due to total temporary anaemia of the central nervous system. *J. exp. Med.*, 1909, **11**, 257-265.
- GRENELL, R. G. Central nervous system resistance: I. The effects of temporary arrest of cerebral circulation for periods of two to ten minutes. *J. Neuropath. exp. Neurol.*, 1946, **5**, 131-156.
- GUTHRIE, E. R., & HORTON, G. P. *Cats in a puzzle box*. New York: Kinehart, 1946.
- HAMILTON, H. L. *Lillie's development of the chick*. New York: Holt, 1952.
- HARLOW, H. F. Primate learning. In C. P. Stone (Ed.), *Comparative psychology*. (3rd ed.) New York: Prentice-Hall, 1951. Pp. 183-238.
- HIMWICH, H. E. *Brain metabolism and cerebral disorders*. Baltimore: Williams & Wilkins, 1951.
- HIMWICH, H. E., & FAZEKAS, J. F. Comparative studies of the metabolism of infant and adult dogs. *Amer. J. Physiol.*, 1941, **132**, 454-459.
- HURDER, W. P. Quantitative cortical changes produced by anoxia in rats. *J. comp. physiol. Psychol.*, 1952, **45**, 362-367.
- HURDER, W. P., & SANDERS, A. F. The effects of neonatal anoxia on the maze performance of adult rats. *J. comp. physiol. Psychol.*, 1953, **46**, 61-63.
- INGALLS, T. H. Etiology of mongolism. *Amer. J. Dis. Child.*, 1947, **73**, 147-165.
- INGALLS, T. H. Pathogenesis of mongolism. *Amer. J. Dis. Child.*, 1947, **73**, 279-292.
- JASPER, J. H., BRIDGMAN, C. S., & CARMICHAEL, L. An ontogenetic study of cerebral electrical potentials in the guinea pig. *J. exp. Psychol.*, 1937, **21**, 63-71.
- JOHNSON, P. O. *Statistical methods in research*. New York: Prentice-Hall, 1949.
- JONES, L. V. Tests of hypotheses: One-sided vs. two-sided alternative. *Psychol. Bull.*, 1952, **49**, 43-46.
- KENDALL, M. G. *Rank correlation methods*. London: Charles Griffin, 1948.
- LEVINSON, BILLEY. Effects of fetal irradiation on learning. *J. comp. physiol. Psychol.*, 1952, **45**, 140-145.
- LIBET, B., FAZEKAS, J. F., & HIMWICH, H. E. The electrical response of the kitten and adult cat brain to cerebral anemia and analeptics. *Amer. J. Physiol.*, 1941, **132**, 232-238.
- LICKLIDER, J. C. R., & BUNCH, M. E. Effects of enforced wakefulness on growth and maze learning performance of white rats. *J. comp. physiol. Psychol.*, 1946, **39**, 339-350.
- LITTLE, W. J. On the influence of abnormal parturition, difficult labours, premature birth, and asphyxia neonatorum on the mental and physical condition of the child, especially in relation to deformities. *Trans. obstet. Soc. Lond.*, 1861, **3**, 293-344.
- MCNEMAR, Q. *Psychological statistics*. New York: Wiley, 1949.
- MEIER, G. W. Delayed effects of natal anoxia upon behavior and electroencephalographic activity. Unpublished doctoral dissertation, Washington University, St. Louis, 1953.
- MEIER, G. W. Prenatal anoxia in relation to behavioral phenomena in other animals. In W. F. Windle (Ed.), *Neurological and psychological deficits in asphyxia neonatorum*. Springfield, Ill.: Charles C. Thomas, in press.
- MEIER, G. W., & BUNCH, M. E. The effects of natal anoxia upon learning and memory at maturity. *J. comp. physiol. Psychol.*, 1950, **43**, 436-441.

- MEIER, G. W., & MENZEL, E. W. Prenatal oxygen deprivation and subsequent behavior dysfunctions. *Science*, 1955, **122**, 419-420.
- MOSES, L. E. Non-parametric statistics for psychological research. *Psychol. Bull.*, 1952, **49**, 122-143.
- PALMER, F. H. Comparative performance of cats and rats in double alternation problem of the temporal maze. Paper read at Midwest Psychological Association, Detroit, 1950.
- PRESTON, MARY I. Late behavioral aspects found in cases of prenatal, natal, and postnatal anoxia. *J. Pediat.*, 1945, **26**, 353-366.
- ROSENFELD, G. B., & BRADLEY, C. Childhood behavior sequelae of asphyxia in infancy, with special reference to pertussis and asphyxia neonatorum. *Pediatrics*, 1948, **2**, 74-84.
- ROYCE, J. R. The factorial analysis of animal behavior. *Psychol. Bull.*, 1950, **47**, 235-259.
- SCHEIDLER, C. H. The effects of prenatal anoxia on learning of white rats. Unpublished doctoral dissertation, Washington University, St. Louis, 1953.
- SCHREIBER, F., & GATES, N. Cerebral injury in the newborn due to anoxia at birth. *J. Mich. State med. Ass.*, 1938, **37**, 145.
- SEWARD, J. P. The sign of a symbol: A reply to Professor Allport. *Psychol. Rev.*, 1948, **55**, 277-296.
- SIEGEL, S. *Nonparametric statistics*. New York: McGraw-Hill, 1956.
- SNYDER, F. F. *Obstetric analgesia and anesthesia*. Philadelphia: Saunders, 1949.
- VAN LIERE, E. J. *Anoxia: Its effect on the body*. Chicago: Univer. Chicago Press, 1942.
- WINDLE, W. F., & BECKER, R. F. Asphyxia neonatorum. An experimental study in the guinea pig. *Amer. J. Obstet. Gynec.*, 1943, **45**, 183-200.
- WINDLE, W. F., BECKER, R. F., & WEIL, A. Alterations in brain structure after asphyxiation at birth. An experimental study in the guinea pig. *J. Neuropath. exp. Neurol.*, 1944, **3**, 224-238.
- YOUNGDAHL, PATRICIA L. The effects of natal anoxia on the learning ability of white rats. Unpublished master's thesis, Washington University, St. Louis, 1948.

(Received October 17, 1958)

APPENDIX A

ELECTROENCEPHALOGRAPHIC DATA

Even though the analysis of electroencephalographic frequencies and amplitudes for differences in the normal and natively anoxic groups of cats proved negative, certain regularities appeared in both groups as related to the development of electrical activity of the brain.

The frequencies of the EEGs through maturation typically increased until the third or fourth month when the adult level was reached. At birth the dominant frequency was less than 1 cycle per second (cps) with occasional "runs" of greater frequencies. At the end of the first month, 3-5 cps activity was typical; at the end of the second month, 5-7 cps; and at the end of the third or fourth month, 6-9 cps. These last frequencies were those found at the end of the sixth month and, again, at the final recording: 7-9 cps activity was representative of the frontal leads and 7-8 cps of the occipital.

This listing of "dominant" frequencies should not be construed to mean that these were the only frequencies seen at the particular recording. The spectra of frequencies were much the same for all ages, that is, both slow (1-2 cps) and fast (10-15 cps) appeared in the records at all ages. The frequencies listed as dominant are those which appeared most frequently in the record of a given *S* for a given age. This does not imply, further, that these frequencies constituted necessarily the majority of the record.

Dominance appeared in the occipital leads beginning with the third recording (end of the second

month). Almost universally, the left occipital records had greater amplitudes than the right.

As indicated earlier (cf. *Apparatus and Procedure*) the electroencephalographic recordings at the time of deprivation demonstrated a pattern of activity correlated with the severity and duration of anoxemia. Prior to the deprivation the frequencies were typically less than 1 cps within frequent sequences of 3-5 cps activity. During the earlier stages of deprivation, about the stage when the equivalent oxygen in the chamber was reduced by half, the frequencies increased and regular sequences of 3-5 cps activity prevailed. This was concurrent with increased motor activity of the *S*; the increased electrical activity appeared to be causally related to motor action. After this point, the frequency and amplitude diminished continuously until the deprivation bout was concluded. From many of the *S*s, no activity could be recorded at this time; that is, by this instrumentation both the frequency and the amplitude were reduced to "zero" (5 microvolts produced $\frac{1}{16}$ in. deflection). Occasionally, however, some of these periods of "no activity" were disturbed by high amplitude (high frequency bursts that were coincidental with gasping behavior), the last reflex to be abolished. Whether any relationship existed between the "absence" of electrical activity (rather than a mere diminution of frequency and amplitude) at this time and defective behavior later could not be discerned from the data collected in this study.

APPENDIX B

HISTOLOGICAL TECHNIQUE AND RESULTS

The preparation of the material for histological examination of permanent changes attributable to prenatal anoxia was as follows:

The animals were first anesthetized (40 mg. pentobarbital/kg. body wt., administered intraperitoneally) and then killed by perfusion with an isotonic saline solution followed by a 10% solution of formaldehyde.

The brains were removed and stored for at least two weeks in the 10% formaldehyde solution before preparation for sectioning was begun. Save for the cerebellum with the attached pons and medulla, the brain was cut transversely to the long axis, perpendicular to the surface of the cerebrum. For the purposes of this analysis, two consecutive sections (cut at 10 micra) out of each 16 were mounted and stained with thionin, a Nissl stain. However, only the sections through the cerebrum in the region of the thalamus were examined.

The rationale for this procedure was founded on certain facts of neuronal degeneration and on certain findings reported by other investigators. In all instances in this study, the deprivation of oxygen occurred at least four months prior to the removal of the brain. This meant that any change in the morphology of the individual cells would, in all likelihood, be corrected, or, if not, the cell would have died and complete degeneration taken place with the subsequent removal of the debris. Other investigators, notably Hurder (1952), have reported that the significant neural difference between animals exposed to anoxia and their controls was in the number of neurones per unit of cortical tissue. Although some evidence indicates the possibility of localized and diffuse lesions (Windle et al., 1944), it seemed, from what is known of the degenerative process and from the empirical data collected in an experiment similar to the present one, that the best procedure would be to prepare the tissues for cell counts. This, however, would not necessarily limit the possibilities for locating and identifying lesions and other gross anomalies.

TECHNIQUE

For analysis, four sections, free of artifacts and clearly perpendicular to the surface of the brain, were selected from each animal. These sections were as widely distributed as was possible while still complying with the above limitations.

Before any quantitative analysis was made, the sections were first observed for structural and cellular abnormalities, tumors, variations in apparent size of cells, and evidence of recent trauma.

The comparisons between the two groups regarding cell number proceeded along two lines. It

was first recognized that as a consequence of oxygen deficiency, some of the cortical cells could be lost. In the adult tissue, however, the loss could be signified by a reduction in the thickness of the cerebral cortex or by a reduction in the number of neurones per unit of cortex. As the rats were in the fetal stage at the time of deprivation, both indications of reduction in the absolute number of cells were likely. Consequently, attempts were made to determine both with this material.

The thickness of the cortex was measured with an eyepiece micrometer (with a magnification of 35 \times) at five pairs of corresponding points on either side of the sections.

For the estimation of relative cell density, counts were made on 25 fields, randomly selected, from 4 sections. At a magnification of 450 \times , the counts were made as follows: using an eyepiece equipped with one short and four long pointers, the observer counted the number of pyramidal cells (as indicated by the appearance of the nucleus on the particular section at the particular level of focus) in layers III and V covered by the tips of the four long pointers. (The short pointer was used to focus the field.) It was possible, thereby, that within a single field as many as 4 or as few as 0 could be so covered by the tips. For the 25 fields, 100 cells could be so covered—or, possibly, none. The number of such "hits" was the datum for the individual *S* for intersubject and intergroup comparisons.

The reliability of this procedure was checked with material prepared in the same fashion as this but taken from animals other than those used in this experiment. These results are given in Table 27. A coefficient of concordance (Kendall's *W* [Siegel, 1956]) computed on these counts was .59 which is significant at the .05 level.

TABLE 27

RELIABILITY CHECK ON TECHNIQUE FOR CELL COUNT

Section and Slide	Count					Mean
	1	2	3	4	5	
1	18	17	14	19	16	16.8
2	15	14	13	14	16	14.4
3	18	16	19	19	17	17.8

Note.—The material for this reliability check was taken from a single animal, a normal adult female rat.

RESULTS

The qualitative examination of the tissues revealed no significant structural difference between the experimental and control animals. The size and shape of the cells were uniform in both groups of animals. The only pathology which could not be attributed to artifact was a glial module in one of the female control "dulls."

In estimating absolute cell number from the thickness of the cortex, the observers found greater within subject variability than between subject variability. The measurements differed more with the portion of the brain from which the section was taken than from one *S* to another. (In some instances, unfortunately, the sections from the con-

trol and experimental animals were not taken from corresponding portions of the brains—even though the original stipulation that the selection be through the region of the thalamus was fulfilled.) The range of thicknesses was from 50 to 110 units.

Finally, as can be noted in Table 28, no expected differences were discerned on the basis of relative cell counts. As it turned out, the counts for the experimental animals were higher than the counts for the comparable control groups. The means were: "bright" controls: 18.4; "bright" experimentals: 19.9; "dull" controls: 14.7; and "dull" experimentals: 17.6. Any statistically significant differences here would be contrary to the predicted findings.

TABLE 28

COMPARATIVE DENSITIES OF LAYERS III AND V OF CONTROL AND EXPERIMENTAL GROUPS

Group	Animal	Sex	Layer	Count	Mean III	Mean V	Mean Total
Bright Control	49	M	III	16.7	17.8	19.0	18.4
			V	14.5			
	81	M	III	19.0			
			V	23.5			
Bright Experimental	42	F	III	17.0	22.4	17.5	19.9
			V	17.0			
	36	F	III	23.0			
			V	16.2			
	48	M	III	24.2			
			V	12.5			
Dull Control	85	M	III	25.2	15.8	13.6	14.7
			V	24.2			
	21	F	III	14.5			
			V	13.7			
	39	F	III	16.1			
			V	12.6			
Dull Experimental	43	F	III	17.0	18.0	17.1	17.6
			V	15.5			
	20	M	III	15.5			
			V	12.8			
	38	F	III	22.2			
			V	21.5			
	62	F	III	19.7			
			V	14.5			
	40	M	III	19.0			
			V	17.0			
	76	M	III	17.1			
			V	17.2			
	80	M	III	17.3			
			V	17.1			
	88	M	III	12.5			
			V	15.5			

from
ough
be
ed.)
nits.
cted
ative
the
unts
eans
peri-
full"
cant
cted

n
al

4

9

7

6

L

4

S

50

1

